

EXPERIMENTAL STUDIES OF SMALL INTESTINAL
FUNCTION AND NUTRITION IN UREMIA

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Malmö 1982

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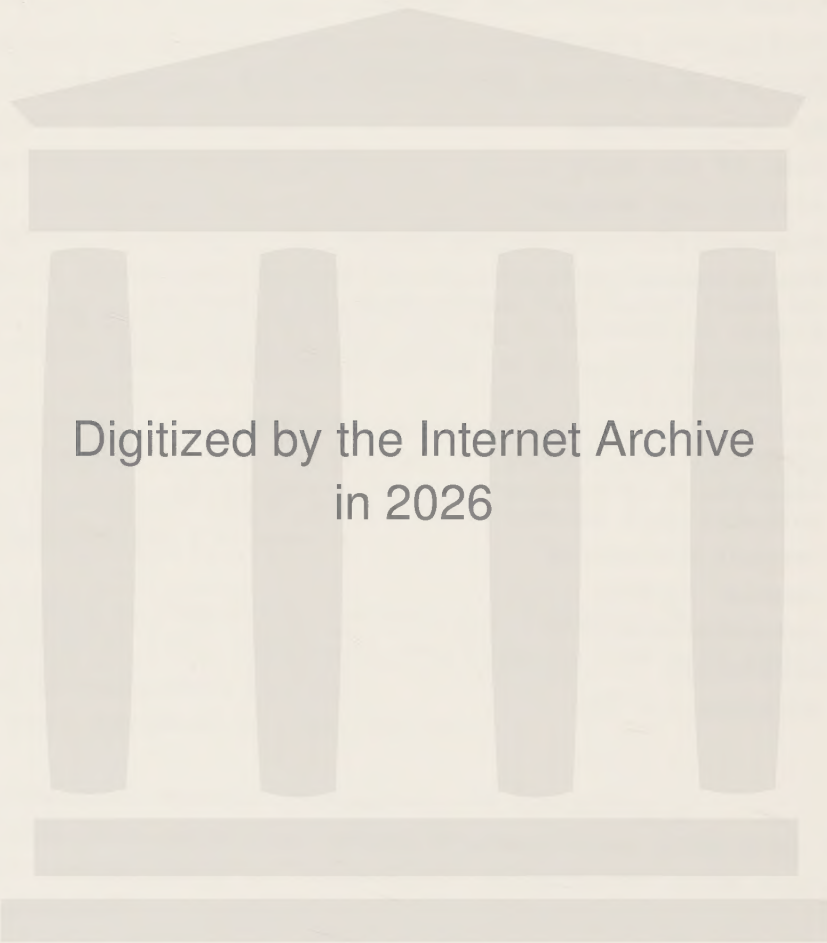
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This thesis is based on the following papers, referred to by their Roman numerals:

- I. Sterner G.
Experimental chronic renal failure in rats.
Nephron 24: 207-208, 1979.
- II. Sterner G, Asp N-G, Dahlqvist A, Denneberg T & Lindberg T.
Small intestinal dipeptidases and disaccharidases in experimental uremia in rats.
Nephron 26: 149-152, 1980.
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Plasma free amino acid levels in uremic rats given high and low protein diets or intravenous infusions of amino acid solutions.
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- VII. Sterner G, Lindberg T & Denneberg T.
Small intestinal absorption of glycine and glycyl-glycine in patients with chronic renal failure.
Submitted for publication.

Contents

INTRODUCTION	7
PREVIOUS INVESTIGATIONS	
Normal function of small intestinal mucosa	8
Uremic small intestinal mucosa	11
Nutritional treatment in uremia	13
Methods of inducing experimental uremia	15
PRESENT INVESTIGATIONS	
Aims of the study	17
Statistical methods	17
Creation of renal failure in rat (I)	17
The influence of uremia and of dietary protein on small intestinal dipeptidase and disaccha- ridase activities in the rat (II, III)	19
Absorptive function of uremic intestinal mucosa in the rat (IV)	21
Total parenteral nutrition in experimental uremia in rat (V, VI)	22
Absorption of glycine and glycyl-glycine in patients with chronic renal failure (VII)	25
General discussion	26
General summary	32
Acknowledgements	33
References	34
Articles I - VII	



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INTRODUCTION

Gastrointestinal complaints, such as nausea, vomiting, and diarrhoea, constitute an important part of the uremic syndrome and usually precede other symptoms as kidney function declines. Morphological changes in the gastrointestinal tract are not uncommon. Mason (81) studied protocols from autopsies on 265 patients with terminal uremia and found in 60% mucosal alterations, such as edema, hemorrhages, ulcers, and necrotic lesions. The changes were most frequently noted in the esophagus, stomach, and colon; a few lesions were also found in the jejunum and ileum.

Histopathological and enzymatical abnormalities have been reported in the small intestinal mucosa in patients with chronic renal failure (CRF) (19, 38, 80, 102). It is still unclear whether these changes affect absorption of nutrients.

In CRF, low protein dietary treatment is often instituted to reduce uremic symptoms and to postpone the start of maintenance hemodialysis. This nutritional treatment is often supplemented with oral amino acids or keto acids and sometimes oligosaccharide solutions are added to provide adequate caloric intake. Thus information on digestion and absorption of protein and carbohydrate in uremia is of importance to optimize therapy in this group of patients.

Experimental renal failure in animal offers a possibility to investigate the influence of uremia on small intestinal function under standardized conditions.

PREVIOUS INVESTIGATIONS

Normal function of small intestinal mucosa

Protein digestion and absorption

Degradation of dietary proteins by pancreatic enzymes result in a mixture of amino acids and peptides of different length in the proximal jejunum. Adibi and Mercer (5) found that the concentration of peptide amino acids were 4 times larger than of free amino acids in jejunal fluid in normal humans after feeding of 50 g bovine albumin. Brush border of the small intestinal absorptive cells (Figure 1) contain several peptidases capable of further splitting the peptides to amino acids, dipeptides, and tripeptides (110).

Using subcellular fractionation of jejunal biopsies from normal subjects, Nicholson and Peters (93) found 96-98% of activity against dipeptides in the cytosol fraction. For some tri- and tetrapeptides 50% and 20% of the hydrolysing activity was localized to the cytosol with the remainder in the brush border fraction. Similar findings have been reported from experimental studies (99). Thus hydrolysing capacity of the cytosol decreases with increasing chain-length of the peptide.

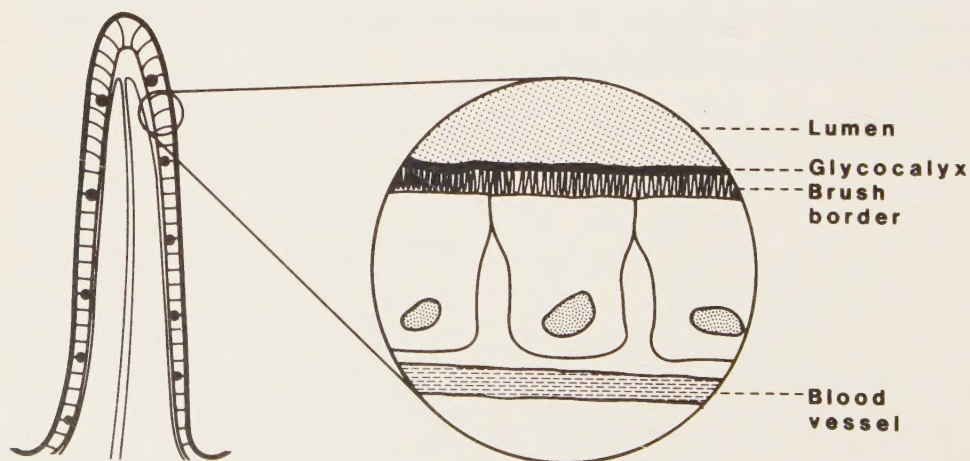


Fig. 1. Small intestinal villus and in magnification the absorptive cells.

Dipeptidases hydrolyse dipeptides containing amino acids in their levo form and are one group out of many of small intestinal peptidases. A substantial part of the 400 possible dipeptides have been tested, and a few separate dipeptidases have been proposed. Two dipeptidases, glycyl-L-leucine and proline dipeptidase, were purified and characterized from pig and monkey small intestine (36, 94, 95, 108, 109). Glycyl-L-leucine dipeptidase has a broad specificity hydrolysing a wide range of dipeptides, whereas proline dipeptidase only splits proline containing dipeptides. Both dipeptidases have been localized to the cytoplasm of the enterocyte using an immunofluorescence method (96). Moreover, a few additional dipeptidases probably exist; glycyl-glycine dipeptidase has been suggested as a separate enzyme (36).

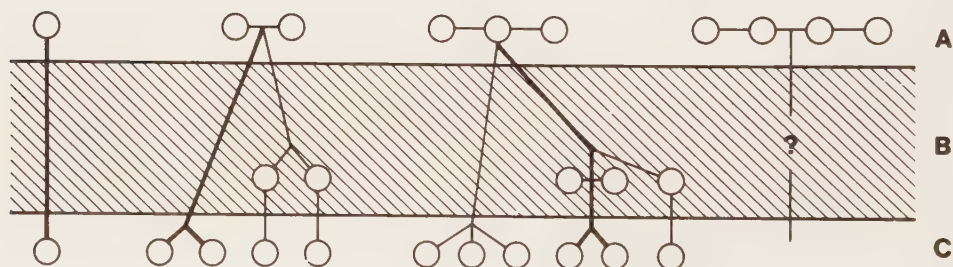
During the first half of this century it was generally believed that digestion in the intestinal lumen continued to complete hydrolysis of the oligopeptides, followed by uptake of the amino acids by the mucosal cells. The turning-point came 1968 when Matthews and Adibi separately showed that, in man and experimental animal, intestinal uptake of amino acids in the form of di- and tripeptides were faster and better than the free amino acids in equimolar concentrations (3, 33, 83). This could not be explained by complete hydrolysis of the peptides before uptake into the mucosa. Their initial studies have been repeated and the results shown valid for several dipeptides in man (4, 54, 106) and rat (24, 29, 84). Different uptake mechanisms were postulated for amino acids and peptides in the intestinal mucosa; some of the evidence coming from studies on patients with Hartnups disease and cystinuria (10, 54, 113).

It has been suggested (85) that the following groups of amino acids are taken up by separate transport mechanisms in the small intestine: monoaminomonocarboxylic (neutral) amino acids, diamino (basic) amino acids, dicarboxylic (acidic) amino acids, and imino acids. Concerning dipeptides, at least two different ways of uptake into the enterocyte have been proposed (86, 107). The quantitatively most important is possibly intact transport of the peptide to the interior of the cell where hydrolysis take place. Alternatively, hydrolysis can occur superficially at the brush border level followed by absorption of the amino acids. The importance of the different uptake mechanisms has been shown to vary

between different dipeptides (87). Tripeptides are hydrolysed to a greater extent by the brush border enzymes before uptake. However, intact absorption of tripeptides have been reported by hamster jejunum in vitro using a hydrolysis-resistant peptide, glycyl-sarcosyl-sarcosine (2). Whether tetrapeptides are taken up in physiological amounts is doubtful, although intact absorption of leucyl-glycyl-glycyl-glycine was suggested from an in vivo study on rat (30). A simplified scheme of the small intestinal handling of amino acids and peptides is given in Figure 2.

Carbohydrate digestion and absorption

Small intestinal digestion and absorption of carbohydrate has been reviewed by Dahlqvist (35). In contrast to dipeptides, it is agreed that most disaccharides are hydrolysed by enzymes localized to the brush border of the small intestinal mucosa and only monosaccharides taken up by the mucosal cell. Some disaccharidases were found to hydrolyse more than one disaccharide; a nomenclature based on the chemical structure of the disaccharide was set up. Accordingly 5 α -glucosidases and 1 β -galactosidase were discovered



A) Lumen

B) Brush border
(Microvillus membrane)

C) Cytosol

Fig. 2. Schematic presentation of small intestinal hydrolysis and absorption of oligopeptides and amino acids.

in the brush border. 4 of the 5 α -glucosidases have maltose hydrolysing activity. However, for convenience, enzyme activity is often named after the substrate. In addition some disaccharidases have been found intracellular, but these enzymes are not considered to take part in the digestion of disaccharides of dietary origin.

Monosaccharides are taken up by a sodium- and energy-dependent transport mechanism in the small intestine. Probably several different carrier systems exist for the monosaccharides.

Uremic small intestinal mucosa

The results from earlier clinical and experimental investigations of the dipeptide and disaccharide splitting activity in the uremic small intestinal mucosa are divergent. Denneberg et al. (38) studied small intestinal biopsies from 17 patients with chronic renal failure; 11 on hemodialysis and 6 conservatively treated. They found that approximately 50% of the patients had reduced glycyl-L-leucine and L-alanyl-L-proline dipeptidase activities; the changes in the other 3 tested dipeptidase activities were less pronounced. Protein intake was approximately 35 g per day in the conservatively treated group and 52 g per day in the group receiving hemodialysis treatment.

A few experimental studies on small intestinal dipeptidases in uremia are available. Grimm et al. (49, 51) used altogether 11 dipeptide substrates and found hydrolysing activity against two, L-leucyl-L-proline and L-methionyl-L-proline, to be slightly higher in the uremic compared to the sham-operated group of animals. No information on protein intake is given in these experimental studies. Wizemann et al. (123) determined dipeptidase activities in intestinal brush border preparations from uremic rats. They found 5 out of 6 enzyme activities to be increased in uremic rats.

Small intestinal disaccharidases in uremia were first evaluated by Madzarovova-Nohejlova (80). She noted a significant decrease of lactase and cellobiase activity in 15 patients with renal insufficiency. Denneberg et al. (38) could not verify this lactase deficiency but found maltase and sucrase activities to be the enzymes

most frequently reduced in uremic patients. However, a few patients had low trehalase, isomaltase, and lactase activities. McNair and Olsen (88), also using small intestinal biopsy technique, found reduced activities of maltase in 4 and of sucrase and lactase in only 2 out of 15 uremic patients.

Grimmel et al. (49, 51) found in experimental studies on uremic rats that sucrase and maltase activities were significantly decreased, whereas lactase and cellobiase were unchanged compared with sham-operated rats. As with the studies on dipeptidases, comparison between the results are difficult, detailed composition of the diets used not being given.

Information on the small intestinal absorptive capacity in uremia is scanty. Gulyassy et al. (52) observed a lower rise of plasma tryptophan after an oral load of the amino acid to patients with end stage renal failure compared with normal subjects. When the experiment was repeated with a synthetic amino acid, having a slow degradation, the difference in plasma levels between uremic and controls was less pronounced. These results indicated that increased metabolism of tryptophan was a more important factor than defective absorption for the low plasma levels of tryptophan in uremic patients. Using the uremic rat model, Kassler et al. (59) found reduced uptake of arginine using the everted sac technique. Wizemann et al. (123) perfused ileal segments of uremic rats and found decreased leucine absorption in moderate uremia and increased absorption of the same amino acid in severe uremia. No works dealing with peptide absorption in uremic intestine have hitherto been published.

Concerning carbohydrate absorption, the finding of low blood glucose levels after oral lactose tolerance tests in uremic patients made by Madzarovova-Nohejlova (80) has not been confirmed by others (19, 50, 88). Bloch et al. (19) found a low-normal glucose uptake in a patient with chronic renal failure using intestinal perfusion. Denneberg et al. (38) noted a lower blood-glucose rise after oral glucose tolerance tests in patients in intermittent hemodialysis compared with normal persons of the same age. Generally a state of glucose intolerance with a delayed fall in blood glucose after oral administration has been reported in uremia (44, 77).

To summarize: Our knowledge of the small intestinal function in uremia is incomplete, and published studies have given divergent results.

Nutritional treatment in uremia

Although the benefit of reducing protein intake to patients with chronic renal failure has been recognized for several decades (22), modern dietary treatment in uremia originated from the findings of Giordano (47). He discovered after a series of metabolic experiments on uremic patients that low nitrogen diet consisting of essential amino acids lowered serum urea and promoted positive nitrogen balance. The findings suggested that excess of urea could be used for anabolic purposes in uremia. Giovanetti and Maggiore (48) applied these results on clinical practice. They treated uremic patients for 3 to 10 months with 10 g protein per day supplemented with essential amino acids or egg protein and noted substantial improvement of gastrointestinal and mental symptoms.

Different variants of the "Giordano-Giovanetti-diet", containing approximately 20 g protein of high biological value, were used in several countries (62, 63, 104). However, poor patient acceptance and progression of peripheral neuropathy was found (63). It was also reported that the 20 g diet was nutritionally inferior to a diet containing 40 g protein per day (64). Bergström et al. (17) introduced a more palatable diet based on 16-20 g of unselected protein supplemented with essential amino acids and including histidine that had been found to be essential in uremia (15, 65). Treatment with this diet was not associated with deterioration of peripheral nerve function (18) or with marked protein malnutrition (12). Although nitrogen balance was positive at the start (11, 17), evaluation after 3 months showed slightly negative balance and alterations in muscle free amino acid concentrations (8). This indicates that amino acid requirements are different in uremia compared with health.

High energy supply, such as fat or carbohydrate, is important when low protein diet is instituted in uremia. If calorie intake is low, amino acids will be used for gluconeogenesis instead of protein synthesis. It was reported that nitrogen balance improved when energy intake increased (57, 60) and at least 35 kcal/kg/day has been recommended in chronic renal failure (66).

Utilization of urea in uremic patients was earlier thought to be substantial and to occur through degradation to ammonia by bacteria in the intestinal lumen followed by reabsorption and transamination to non-essential amino acids in the liver. However,

further studies have not confirmed this hypothesis as the amount of urea reutilized for protein synthesis was found to be low (101). Giordano already in 1963 (47) showed that the urea lowering effect of essential amino acids continued after sterilization of the gut.

The pattern of plasma free amino acids is often altered in patients with CRF (67) as well as in animals with experimental uremia (61, 112). Essential amino acids are generally decreased whereas some non-essential amino acids are increased. To what extent these changes are due to protein malnutrition, to malabsorption or retention of amino acids, or to different metabolism in the liver and kidney is not clear.

When gastrointestinal symptoms prevent oral food intake in patients with acute or chronic renal failure, parenteral nutrition forms an important alternative treatment (1, 20, 40, 56, 72, 121). It was early found that administration of a fat emulsion of soybean oil with egg-yolk phosphatides as emulsifier was well tolerated by the patients (71, 72). Infusion of this fat emulsion facilitated a high energy supply in fluid restricted patients with acute renal failure. However, intravenous administration of fat was not generally accepted because of fear of accelerating the hyperlipemia often found in uremia. Subsequent studies of parenteral nutrition in uremia have been limited to giving amino acids and hypertonic glucose (for review see 20). A frequent finding was that the rise in serum urea and potassium was less with glucose and essential amino acids compared with glucose alone. Abel et al. (1) showed that administration of glucose and essential amino acids to patients resulted in better survival and faster recovery from acute renal failure than giving glucose alone. These findings were supported by experimental results on rats with mercury-chloride-induced acute renal failure (115).

Concerning infusion of amino acids, the optimum quantity and composition is still to be found. Bergström et al. (16) supplemented oral low protein diet with essential amino acids given intravenously and noted improved nitrogen balance. In acute or chronic renal failure, nitrogen requirement is different due to hypercatabolism. Moreover, amino acids losses during dialysis treatment contribute to the negative nitrogen balance found in these patients (46,125). The possible advantage of giving both essential and non-essential amino acids in this situation has not yet been fully evaluated.

To summarize: Oral low protein diet supplemented with essential amino acids has become an important part of the conservative treatment of uremia. As yet, there is lack of information concerning the intestinal uptake of amino acids in uremia. Intravenous nutrition carries certain advantages, but is still not fully evaluated. As clinical studies are hard to interpret, there is need for an experimental model.

Methods of inducing experimental uremia

Experimentally induced renal failure in animals has been widely used for studying different aspects of the uremic syndrome. Bilateral nephrectomy and ureteral ligations produce a severe acute uremia with a survival of only a few days (31, 53). Renal failure of varying duration has been achieved in animals using injection of nephrotoxic substances (45, 74).

The most frequently used methods for inducing chronic renal failure have been either ligation of renal artery branches (13) or surgical removal of renal parenchyma, i.e. partial nephrectomy. Although sheep have been used for experimental uremia when vascular access is important (42) rat has been the most commonly utilized animal.

Chanutin and Ferris (28) were among the first to describe partial nephrectomy as a method for inducing chronic renal failure in rat. They resected the upper and lower poles of the left kidney and removed the right kidney after one week. The rats thus treated developed polyuria, albuminuria, nitrogen retention, hypertension, and cardiac hypertrophy. Platt et al. (100) studied morphological changes and laboratory parameters in rats partially nephrectomized in two seances. Two weeks after the last operation, serum creatinine had doubled and 24-hours creatinine clearance decreased to 55% of the preoperative values. Morrison (91) using the same method as Platt et al. (100) only measured serum urea and found mean values in uremic rats 2 times higher after 17 weeks and 3 times higher after 45 weeks compared with sham-operated animals. Several other authors have used this uremic rat model to study the effects of renal failure on different organ systems (9, 27, 32, 41, 43, 49, 51, 126).

As clearance studies were seldom included in these reports, no definite conclusion can be drawn about the degree of the kidney failure. Judging from the animals weight increase, it is doubtful whether a profound uremia actually existed.

Alam et al. (7) extended the resection to both poles and the lateral parenchyma of the kidney, and Mehls et al. (90) combined partial nephrectomy with local irradiation of the kidney remnant to reduce hypertrophy of the parenchyma. Boudet (23) used electrocoagulation of the renal cortex and thus achieved a longstanding renal failure with creatinine clearance of 10 to 20% of normal. The relatively long time required before the animals become uremic is a disadvantage with the latter method.

PRESENT INVESTIGATION

The aims of the study were:

- To develop an experimental animal model of severe renal failure.
- To study dipeptidase and disaccharidase activities in small intestinal mucosa of uremic rats and the influence of dietary protein on these enzyme levels.
- To investigate the absorptive capacity of the small intestine in experimental uremia.
- To apply total parenteral nutrition to uremic rats and to investigate the effects of different amino acid solutions and of a fat emulsion given intravenously.
- To study absorption of amino acids and dipeptides in patients with chronic renal failure.

Methods and Results

Statistical methods

For values assumed to be normally distributed, mean and standard deviation was used; significance of difference was calculated with Student's t-test or analysis of variances. When the groups were small (less than 10 observations), non-parametric methods, such as Mann-Whitney rank sum test and Wilcoxon test for paired data, were employed. Values were presented as mean (or median) and range. Differences were considered significant when p-values less than 0.05 were obtained.

Creation of renal failure in rat (I)

Method

Different surgical procedures were used to evaluate partial nephrectomy in rats as a method of inducing renal failure. Partial resection of the left kidney followed by contralateral nephrectomy after one week was found to be the preferred method. When the operations were performed in the everted order or in one single seance, the survival of the animals was reduced. Furthermore, only the extensive resection of the left kidney rendered the rats seve-

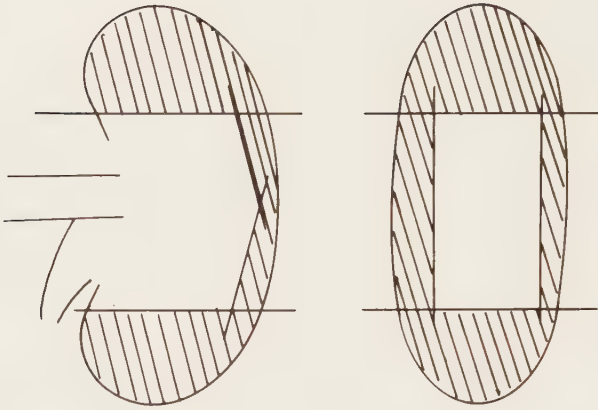


Fig. 3. Resection of the left kidney, anterior and lateral view.

severely uremic. As Figure 3 shows, both poles and the parenchyma on the anterior, posterior, and lateral sides were resected. Hemostasis was performed with 5 to 10 minutes of digital pressure. Local irradiation with 290 rad on each side of the kidney to prevent hypertrophy of the remnant was used according to Mehls et al. (90). Sham-operated and irradiated rats were used as controls.

Results

Partial nephrectomy, as used here with extended resection of the left kidney, resulted in a rapid and profound uremia with an acceptable mortality of less than 10%. The rats did not gain weight during the weeks after the partial nephrectomy, and the fur became sparse and fuzzy. After 5 to 6 weeks their weight dropped and symptoms of terminal uremia developed, such as bleeding and twitching, and the animals died.

Four weeks after the last operation, at the time when most experiments were performed, serum creatinine was increased 7 times and 24-hours creatinine clearance was reduced to 10% of control rats. Serum urea was almost 10 times higher in uremic rats than in sham-operated rats. Other laboratory abnormalities known to be present in severe renal failure were also noted; hyperpotassemia, hypocalcemia, hyperphosphatemia, anemia, and a slight acidosis (Table I).

Table I. Laboratory data in uremic and sham-operated rats (mean $\pm$ SD). Number of rats in parentheses.

	Uremic rats	Sham-operated
Serum-creatinine $\mu\text{mol/l}$	368 $\pm$ 80.3 (19)	51.6 $\pm$ 8.76 (9)
Creatinine clearance, $\mu\text{l/min/100 g b.w.}$	65.1 $\pm$ 25.9 (19)	688 $\pm$ 165 (9)
Serum urea, mmol/l	69.9 $\pm$ 13.4 (12)	7.46 $\pm$ 1.79 (5)
Serum sodium, mmol/l	148 $\pm$ 9.57 (12)	142 $\pm$ 3.42 (7)
Serum potassium, mmol/l	5.44 $\pm$ 0.79 (12)	4.80 $\pm$ 0.31 (7)
Serum chloride, mmol/l	106 $\pm$ 9.80 (12)	104 $\pm$ 4.63 (7)
Serum calcium, mmol/l	1.86 $\pm$ 0.37 (12)	2.37 $\pm$ 0.15 (7)
Serum phosphate, mmol/l	3.98 $\pm$ 0.80 (12)	2.70 $\pm$ 0.23 (7)
Serum magnesium, mmol/l	1.32 $\pm$ 0.27 (12)	1.24 $\pm$ 0.18 (5)
Plasma bicarbonate, mmol/l	19.0 $\pm$ 1.94 (9)	20.8 $\pm$ 0.45 (5)
Hematocrit, %	32 $\pm$ 1.69 (8)	48.4 $\pm$ 1.52 (5)

The influence of uremia and of dietary protein on small intestinal dipeptidase and disaccharidase activities in the rat (II, III)

Methods

Enzyme assays. Enzyme activities were determined in homogenates of small intestinal mucosa of uremic and control rats 4 weeks after the contralateral nephrectomy and sham-operation, respectively. Dipeptidase activities were measured according to Josefsson and Lindberg (58) using glycyl-L-leucine, L-methionyl-L-methionine, and L-alanyl-L-proline as substrates. Disaccharidase activities (substrates: maltose, sucrose, trehalose, and lactose) were determined as described by Dahlqvist (34).

Dietary regimens. Dipeptidase activities were assayed after feeding the rats ad libitum a normal laboratory diet (24% protein), a low protein diet (6% protein) based on egg albumin, and a total parenteral nutrition, including fat, carbohydrate, and amino acids. The nitrogen supplied with the parenteral nutrition was comparable to the low protein diet given orally. Disaccharidase activities were assayed after the two oral diets.

Results

Intake of the oral diets in relation to body weight did not significantly differ between uremic and sham-operated rats. In the uremic group given the 24% protein diet glycyl-L-leucine and L-methionyl-L-methionine dipeptidase activities were significantly increased in jejunal mucosa compared with sham-operated rats (Fig. 4). No corresponding change in the dipeptide splitting activities of liver homogenates from these animals were observed. The uremic rats fed the 6% protein diet had significantly lower glycyl-L-leucine and L-methionyl-L-methionine dipeptidase activities in jejunum than the uremic rats that were given the 24% protein diet. The lowest level for these enzymes were found in uremic rats on total parenteral nutrition. No significant alteration was found in

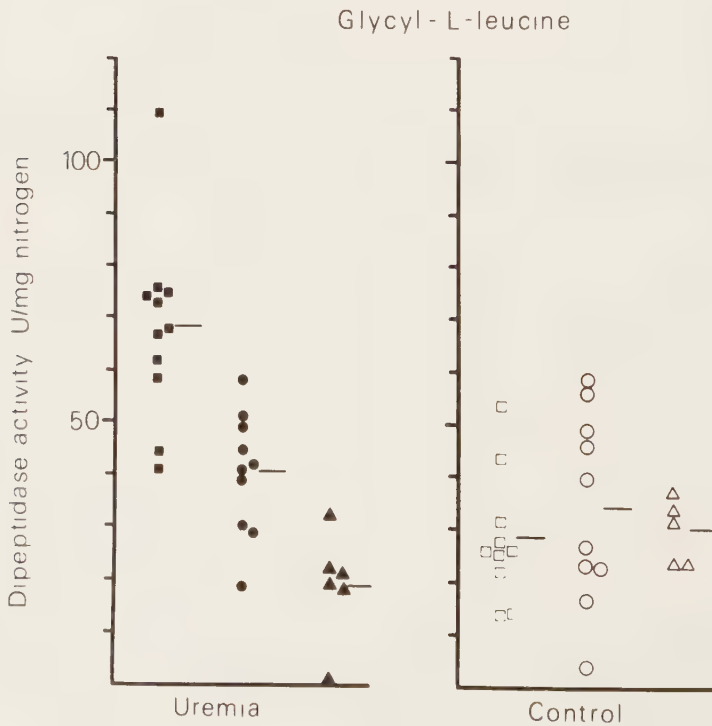


Fig. 4. Glycyl-L-leucine dipeptidase activities in uremic rats (filled symbols) and sham-operated rats (open symbols). $\square$ = 24% protein diet, $\circ$ = 6% protein diet, Δ = total parenteral nutrition.

the jejunal mucosa from sham-operated rats concerning these enzymes. L-alanyl-L-proline dipeptidase activity showed an inverse relation to protein intake in both uremic and sham-operated animals.

Among the disaccharidases, sucrase was significantly increased in uremic rats on both 24% and 6% protein diets compared with sham-operated rats. Lactase activity, on the other hand, was normal in uremic rats on 24% protein diet but increased in the uremic animals given 6% protein diet.

Absorptive function of uremic intestinal mucosa in the rat (IV)

Methods

In vitro absorption was studied with jejunal rings as described by Agar et al. (6). The rings were incubated in physiological buffer solution containing ^{14}C -labelled glycine, L-leucine, glycyl-L-leucine, or glucose. Uptake was measured as radioactivity in the rings after incubation for 5 minutes. The glycyl-L-leucine used was labelled either in the glycine or in the L-leucine. The radioactivity was determined in a Packard-Tri-Carb model 3375 liquid-scintillation spectrometer.

The in vivo study measured the absorption of the amino acids glycine, L-leucine, L-valine, L-lysine, and L-methionine and the dipeptides glycyl-L-leucine, glycyl-L-valine, and glycyl-L-lysine. Solutions containing either the amino acids or the dipeptides were injected into separate jejunal loops on anaesthetized uremic and control rats. PEG (polyethyleneglycol 4000) was used as a non-absorbable marker. After 5 minutes the loops were emptied. Uptake was calculated from the difference in luminal concentration before and after the test, correcting for water transport according to the final PEG concentration. For the quantitative determination of amino acids and dipeptides, an amino acid analyser (Kontron liquimat III) was used.

Results

The uptake of labelled amino acids, dipeptide, and glucose in vitro did not differ between uremic and control rats. For both uremic and sham-operated rats, L-leucine was absorbed better than glycine when tested as free amino acids. This difference was not

present when the amino acids were presented as the dipeptide, glycyl-L-leucine. Thus glycine was taken up 3 times better in the dipeptide form than as free amino acid.

In vivo it was found that severely uremic rats, with a serum creatinine of more than 250 $\mu\text{mol/l}$, had a reduced ability to absorb some amino acids compared with non-uremic rats (Fig. 5). No corresponding decrease in the uptake of dipeptides was found with increasing uremia.

Total parenteral nutrition in experimental uremia in rat (V, VI)

Methods

A technique for the administration of total parenteral nutrition (TPN) to animals earlier described (103) was used in the uremic rats. The total infusion volume was adapted to the ad libitum fluid intake of the uremic rats. The parenteral nutrition included a 50% glucose solution, a 20% fat emulsion, and either a "complete" amino acid solution (CAA) with essential and non-essential amino

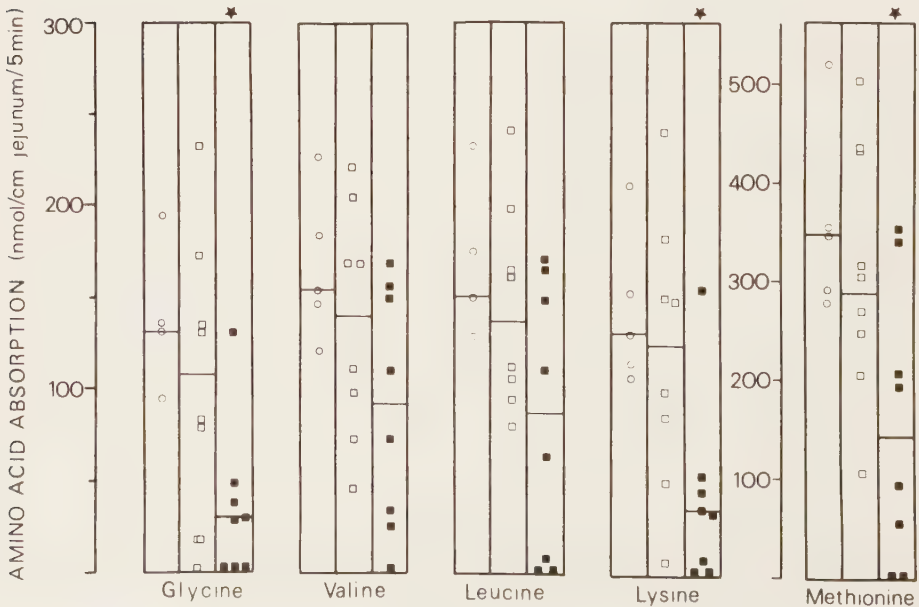


Fig. 5. In vivo uptake of some amino acids in sham-operated rats (o), moderately uremic rats (□), and severely uremic rats (■).

acids or a solution containing only the essential amino acids plus histidine (EAA). A specially composed vitamin and mineral solution was included to provide a complete nutrition according to the requirements of the rat. Weight gain, nitrogen balance, and nitrogen utilization were measured during the study and compared to rats fed a 24% and 6% protein diet orally. At day 0, 2, and 10, plasma free amino acids and other blood biochemical data were determined. Nitrogen was analysed according to a micro-Kjeldahl technique (114). Plasma amino acids were analysed by ion-exchange chromatography on a Durrum D-500 Amino Acid Analyser.

Results

TPN was well tolerated and 17 of 25 uremic rats and 12 of 17 sham-operated completed the experimental period. Uremic rats on TPN with CAA and EAA showed positive nitrogen balances, and their weight increases were similar to those found in the sham-operated groups. Uremic rats given the oral diets increased only 1.8 g/day in spite of positive nitrogen balances. Nitrogen utilization was high in all groups given TPN, although significantly lower in uremic rats given TPN with CAA compared with controls.

Plasma urea dropped significantly during TPN and 6% protein oral feeding in uremic and sham-operated rats, but remained high in the 24% protein fed uremic rats. In uremic rats, serum creatinine dropped significantly after TPN with either EAA or CAA, remained unaltered during 6% protein diet, and increased significantly during 24% protein diet. 24-hours creatinine clearance was measured in a few uremic rats and a decrease was noted in rats fed the two oral diets, though significant only for the 6% protein diet. Creatinine clearance was similar at day 0 and day 10 in the parenteral nutreated uremic groups. Hemoglobin and serum albumin were reduced in all uremic groups irrespective of type of nutrition given.

Reduced levels of essential amino acids and increased concentrations of some non-essential amino acids were found in uremic rats given oral feeding. The plasma levels of citrulline, glycine, hydroxyproline, and 1- and 3-methylhistidine were elevated in all uremic rats. Plasma tryptophan was reduced in 3 or 4 uremic groups. The branched-chain amino acids were reduced in plasma of uremic rats given 24% and 6% protein diet orally but not after intrave-

nous infusion. Plasma tyrosine level was reduced in the two uremic groups orally fed, but increased in uremic rats given essential amino acids intravenously. 1- and 3-methylhistidine levels were significantly increased in uremic rats at the start of the treatment. The uremic rats fed the 24% protein diet did not show any change in the levels of 1- and 3-methylhistidine, whereas these metabolites decreased rapidly after the 6% protein diet and TPN. Figure 6 gives different amino acid ratios calculated for the nutritional treatments.

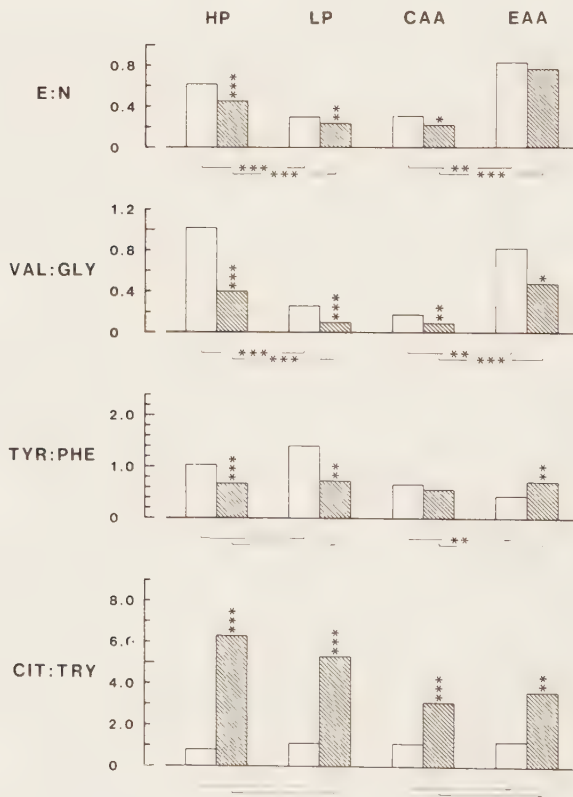


Fig. 6. Plasma amino acid ratios in control (□) and uremic rats (▨) on different treatment. E/N = essential to non-essential amino acids. HP = oral high protein diet, LP = oral low protein diet, CAA = complete amino acid solution, EAA = essential amino acid solution.

Absorption of glycine and glycyl-glycine in patients with chronic renal failure (VII)

Material and Methods

Glycine and glycyl-glycine was given as oral tolerance tests after an overnight fast to 9 patients with chronic renal failure (mean creatinine clearance 9 ml/min) and to 6 healthy subjects. The amino acid or the dipeptide was dissolved in 250 ml water and given with 2 to 7 days interval to the patients and controls. The quantities of glycine and glycyl-glycine were chosen so that the number of glycine units were the same in both solutions. Blood was taken before the test and at 15, 30, 45, 60, 90, and 120 minutes after the administration. Plasma was analysed for α -amino nitrogen according to a method modified by Matthews et al. (82). Curves representing the uptake of glycine and glycyl-glycine were drawn, and the area under curve (AUC) was calculated.

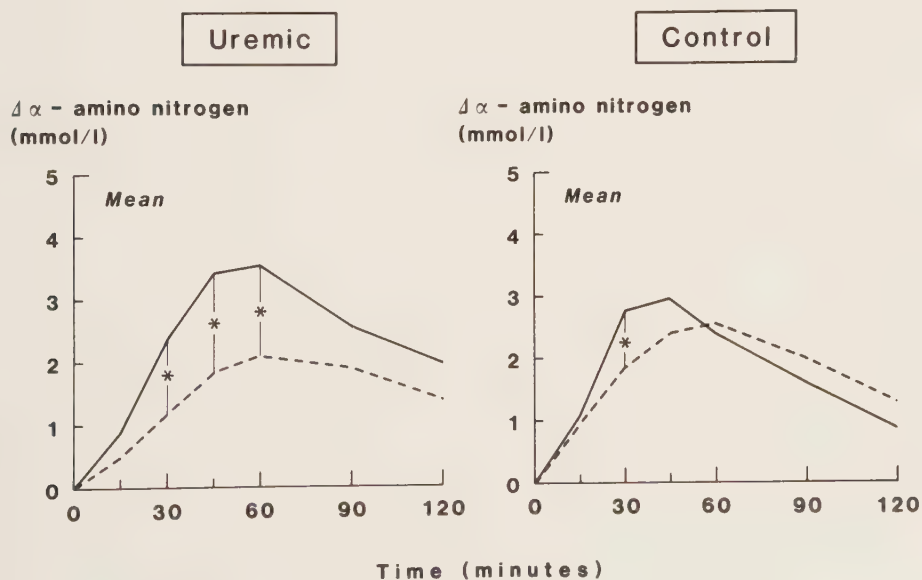


Fig. 7. Mean plasma α -amino nitrogen after administration of glycine (-----) and glycyl-glycine (——) to uremic patients and controls.

Results

Plasma α -amino nitrogen in uremic patients was significantly lower after glycine administration than after glycyl-glycine at 30, 45, and 60 minutes. Also the AUC was smaller for the amino acid than for the dipeptide in the uremic group, indicating a lower total uptake. One patient with terminal renal failure and a creatinine clearance of only 3 ml/min had a flat plasma curve after glycine administration, whereas the glycyl-glycine curve remained almost normal.

In the control group, plasma α -amino nitrogen was lower at 30 minutes after the glycine than after the glycyl-glycine administration, although AUC did not significantly differ. Figure 7 shows the mean plasma values after giving the amino acid and dipeptide to uremic patients and controls.

General discussion

Partial nephrectomy in rat has become a frequently used method to induce experimental chronic renal failure. With the present technique, using an extensive resection and local irradiation of the left kidney, it was possible to reduce kidney function, as measured by 24-hours creatinine clearance, to 10% of that of sham-operated rats. A similar surgical approach was recently reported by Ormrod and Miller (97). They found that it was possible to attain varying levels of uremia by resecting different amounts of renal parenchyma. As in the present investigation, the most severely uremic rats in their study had a glomerular filtration rate of approximately 10% of normal. The reduced growth and the abnormal laboratory parameters, including the plasma amino acid concentrations found in the rats in our study, agree with that observed in patients with a severe uremic state.

The main purpose of this experimental study was to investigate the small intestinal function in uremia. Earlier experimental and clinical data suggest that a gastrointestinal and pancreatic dysfunction exist in uremia (14, 50, 78, 122). This could have implications on the nutrition of patients with chronic renal failure. Morphological changes, such as shortening of villi and elongation of crypts, have been found in the small intestinal mucosa of pa-

tients with varying degree of renal failure (19, 38, 102). In long term experimental uremia in rats, increased mitotic cycle time and increased number of crypt cells were found (79). It has been suggested that in longstanding uremia the crypts adapt to the inhibition of cell differentiation by increasing the cell number (79). Of interest in this connection is the in vitro finding of decreased incorporation of phenylalanine into cell membranes of the intestinal mucosa of uremic rats reported by McVicar et al. (89). Like Grimm (51) the present investigation found no morphological changes in the rat small intestine using ordinary histological preparations, but the duration of the uremia might have been too short. However, in some rats with pronounced uremia, the intestinal wall was edematous and the lumen was filled with dark fluid (unpublished observation).

Concerning small intestinal enzymes, earlier studies using uremic rats fed a diet with a normal protein content have reported increased dipeptide splitting activities (49, 51, 123). The data presented here indicate that in uremia the activity of glycyl-L-leucine dipeptidase, responsible for the hydrolyse of a wide range of dipeptides, depends on protein content in the diet. When the uremic animals were given a diet with a 24% protein content, the enzyme activity towards glycyl-L-leucine was increased, whereas the rats fed a 6% protein diet had a significantly lower enzyme activity. L-methionyl-L-methionine dipeptidase activity behaved similarly; this dipeptide is probably split by the same enzyme as glycyl-L-leucine (36). Total parenteral nutrition further reduced these enzyme activities in uremic rats. This agrees with the findings of reduced intestinal mass and enzyme activities during parenteral nutrition in non-uremic rats (25, 73).

The cause of the increased enzyme activities in uremic rats after feeding a diet with a normal protein content is not clear, but could be explained by an increased content of di- and oligopeptides in the intestinal lumen. Thus it is possible that intestinal dipeptidase activities adapt to variations in the luminal content of peptides. When a low protein diet is given, the content of dipeptides in the intestinal lumen decreases and the dipeptide splitting activity is reduced. Earlier investigations on non-uremic rats given low protein diet have shown reduction of brush border and also cytosol peptide hydrolase activities (70, 92, 111).

In the present study, sucrase activities were slightly increased in uremic rats on a diet with normal protein content. This is contrary to Grimm et al. (49, 51) who found reduced sucrase and maltase levels in the small intestine of uremic rats. Possibly different intake and composition of carbohydrate in the diets used account for these discrepancies. Small intestinal sucrase and maltase activities in normal rat have been found to be regulated by the content of sucrose and maltose in the diet (39). After a low protein feeding, we found significantly higher lactase activities in uremic rats and slightly, though not significant, increased levels in controls. Increased lactase activities have earlier been reported in poorly nourished and low protein fed non-uremic rats (70, 116). In the present study, a similar change was noted for the cytosol enzyme L-alanyl-L-proline dipeptidase.

If the results from the experimental studies on dipeptidase activities are applicable to patients with chronic renal failure, it is possible that the reduced small intestinal dipeptidase activities found by Denneberg et al. (38) were caused by the low protein diet and not by the uremic state per se. It is tempting to suggest that the adaptation of intestinal peptidase to dietary protein could be of practical importance for uremic patients; e.g., when changing from low to high protein diet, from amino acid to protein diet, and from parenteral to oral nutrition.

Concerning the absorptive function of the small intestine in uremia, the results in vivo indicate that in severe uremia the uptake of some amino acids is more depressed than the uptake of corresponding dipeptides. A similar difference between the uptake of amino acids and dipeptides has been found after dietary changes in non-uremic animal and in certain diseases in man (87). The results from earlier investigations on small intestinal uptake of amino acids in uremia are far from clear. Both decreased (59, 123, 124) and increased (89, 123, 124) uptake of amino acids have been reported in studies of uremic rats. Varying levels of uremia and different experimental designs make the comparison between the studies difficult. McVicar et al. (89), using an intestinal perfusion technique in vivo, found the uptake of tyrosine, phenylalanine, and glucose, but not histidine, increased in uremic rats compared to pair-fed sham-operated rats. The perfusion lasted 1 hour and 45 minutes on each rat, and this extended experimental

situation might have damaged the intestinal mucosa. No information was given about the histological appearance of the small intestine after the perfusion test. The amino acids used by McVicar et al. (89) were not studied in the present investigation. However, glucose was included in our in vitro study and no difference was seen between the uptake in the uremic and control group. Rats were not pair-fed in the present investigation and although relative food intake per kg body weight was identical between the groups, total food intake per day was lower among the uremic rats. Earlier reports on short term dietary restriction in non-uremic rats suggest that amino acid uptake is increased in the small intestine (75, 76). Thus it is unlikely that the reduced uptake of amino acids in uremic rats found in our study was caused by dietary factors.

In both the in vitro and in vivo tests, sham-operated rats absorbed the dipeptides slightly better than the corresponding amino acids. This agrees with previous findings (86) and support the existence of independent uptake mechanisms for amino acids and peptides. In the clinical investigation, the small intestinal uptake of glycyl-glycine was significantly better than the uptake of glycine in uremic patients. A similar result, although less marked, was found in the control group. For the uremic group as a whole, uptake of the amino acid was not reduced compared with controls. Although the studied group was small, the results suggest that no severe malabsorption of amino acids or dipeptides takes place in patients with a stable chronic renal insufficiency. However, one of the patients investigated had a terminal renal failure with severe uremic symptoms. Uptake of glycine in this patient was markedly depressed and substantially lower than the uptake of glycyl-glycine. It is possible that further investigations of patients with severe uremic symptoms will reveal that the absorptive capacity for amino acids is impaired.

A better uptake of amino acids in the form of dipeptides than as free amino acids in uremic patients could have nutritional implications. A diet consisting of di- and oligopeptides is less hypertonic than a corresponding amino acid containing diet and would therefore be less prone to cause diarrhoea and other gastrointestinal symptoms. So far, the cost makes the use of such a diet unfeasible in clinical practice.

The data from the total parenteral nutrition show that this is

a useful experimental model and can be used for further studies of intravenous administration of nutrients in uremia. Both the essential and complete amino acid solution used in this study promoted weight gain and positive nitrogen balances. The findings of Pennisi et al. (98) were not confirmed, indicating better weight gain with a mixture of essential and non-essential amino acids than with only essential amino acids given orally to uremic rats. Arginine is considered essential for growing rats (21) and the renal conversion of citrulline to arginine is reduced in uremic rats (26) indicating that arginine should be critical for growth in rats with induced uremia. In spite of this, we did not find any short-term negative effects on weight increase in rats given only essential amino acids without arginine intravenously. Furthermore, Wang et al. (118) reported similar growth rates for uremic rats fed orally an arginine-free diet and a diet containing arginine.

The use of fat emulsions as part of a parenteral nutrition to patients with acute or chronic renal failure was reported by Lee et al. (72). They found that elimination of the emulsified soybean oil was prolonged in patients with chronic renal failure but otherwise the treatment was well tolerated. However, the use of parenteral fat emulsions in uremia has become controversial and was recently not included in a review on nutritional therapy in renal failure (69). The main objection against the use of fat intravenously in renal failure is that it will aggravate the uremic hyperlipidemia, although no studies have confirmed this. This study did not investigate the effect of fat emulsion on lipid metabolism in uremia. However, the administration of fat intravenously to the uremic rats did not cause any complications. Histopathological examination of the rats after 10 days of TPN did not reveal any changes in liver, lungs, and kidney remnant compared with uremic rats on oral nutrition (Kronevi pers. comm.). Moreover, the uremic rats grew better on the intravenous nutrition than on an iso-caloric and iso-nitrogenous oral nutrition. The high content and the type of fat in the TPN could be responsible for this beneficial effect. It could also indicate an impaired uptake function in the small intestine in uremia.

There are several possible explanations, supported by experimental findings, of the abnormal pattern of plasma free amino acids found in renal failure; altered levels of liver and kidney

enzymes responsible for metabolism of amino acids (26, 105, 117, 120), impaired handling of amino acids by the kidneys (68), dietary factors, e.g., protein restriction (119, 126), and hormonal imbalances (37). As discussed above, reduced uptake of amino acids in the small intestine could be a further factor of importance.

The present results indicate that several plasma amino acids and the ratio of essential to non-essential amino acids and also of valine to glycine were affected both by the protein supply and by the reduced renal function. On the other hand, the ratio of tyrosine to phenylalanine and of citrulline to tryptophan were influenced only by the renal failure per se. These experimental findings agree with those reported by Wang et al. (119). The citrulline to tryptophan ratio has not been used earlier, but seems useful, as it constantly reflects only the renal failure. The increased plasma tyrosine and tyrosine to phenylalanine ratio found in uremic rats given TPN with EAA is of particular interest. This finding indicates that other factors than reduced activity of phenylalanine hydroxylase control the plasma level of tyrosine in uremia.

The plasma amino acids measured during TPN reflect the composition of the EAA and CAA solutions. No direct comparison between rats given oral and intravenous nutrition could be made because of different feeding patterns. Further studies using continuous intragastric infusion of amino acids and determination of amino acids in other tissue compartments might be of value.

General summary

Experimental part:

- Partial nephrectomy and local irradiation of the remaining renal tissue in rat is a useful experimental method giving a model simulating the clinical state of severe uremia.
- The levels of small intestinal dipeptidase activities in uremic rats were primarily affected by the protein content in the diet and not by the uremic state. Among disaccharidase activities, only lactase was affected by the dietary protein.
- The uptake of amino acids in the small intestine in vivo was reduced in a group of severely uremic rats. The absorption of the corresponding dipeptides was not similarly affected.
- Administration of total parenteral nutrition to uremic rats promoted a normal weight gain and a positive nitrogen balance and resulted in reduced serum urea and serum creatinine. The infusion of a fat emulsion was well tolerated by the uremic animals. No marked differences in weight gain and nitrogen utilization was found between a mixed amino acid solution and a solution containing only essential amino acids given intravenously.
- Several alterations in the plasma free amino acid levels were found in uremic rats given TPN or oral diets. The amino acid concentrations were affected by both the renal failure and the dietary protein intake.

Clinical part:

- In patients with chronic renal failure (mean creatinine clearance 9 ml/min) glycine was taken up better as dipeptide than as free amino acid. For the uremic group as a whole absorption of glycine did not differ from that of controls. For some severely uremic patients, intestinal uptake of the amino acid was very low whereas absorption of the dipeptide remained normal.

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Experimental Chronic Renal Failure in Rats

Sir,

An experimental model of renal failure would be most valuable for studying metabolism, skeletal changes and intestinal disturbances in uremia. Several authors, i.e. *Platt et al.* [6], *Morrison* [5], *Chantler et al.* [2], and *Ejerblad* [3], have described the so-called 5/6 nephrectomy in rat. The uremia thus achieved has been unsatisfactorily defined. To judge from the increase in body weight, the rats have only been lightly to moderately uremic. *Mehls et al.* [4], by combining 7/8 nephrectomy with local irradiation to inhibit renal hypertrophy, could reduce the endogenous creatinine clearance to 20% of that of controls. *Boudet et al.* [1] describe an interesting method, using electrocoagulation, which results in progressive uremia over several

months. One disadvantage is the difficulty in deciding at the time of operation how uremic the rats will be. I found that extended kidney resection produced a more profound uremia than is shown earlier with partial nephrectomy. 4 weeks after operation creatinine clearance was only 10% of that of normal rats. Below follows a description of the technique.

Wistar rats weighing 150–200 g were anesthetized with mebumal-sodium 60 mg/kg body weight intraperitoneally. The abdomen was opened with a midline incision, the left kidney was separated from the adrenal gland and from perirenal fat, and a clamp was placed on the kidney vessels to minimize bleeding – an earlier problem. After fastening the kidney on a plastic plate, both poles and part of the lateral, anterior, and posterior parenchyma were resected (fig. 1). Special attention was paid to the posterior resection, as the pelvis could easily be damaged. The arterial clamp was removed and hemostasis achieved with 5–10 min digital pressure. The remaining parenchyma was then irradiated with 290 rad on each side. The right kidney was removed 1 week later when the animals had recovered. Weights were taken every week (fig. 2). The uremic rats survived 5–6 weeks after the second operation. Table I lists the laboratory data of the uremic and sham-operated rats. This standardized technique produces an experimental model of severe chronic uremia. Several of the serum abnormalities seen in these rats compare well with those found in patients with chronic renal failure. The uremic model should therefore be useful in studying the effect of uremia on different organ systems.

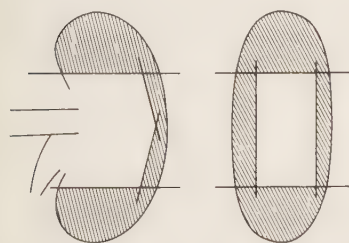


Fig. 1. Resection of left rat kidney, anterior and lateral view. Hatched areas represent resected parenchyma.

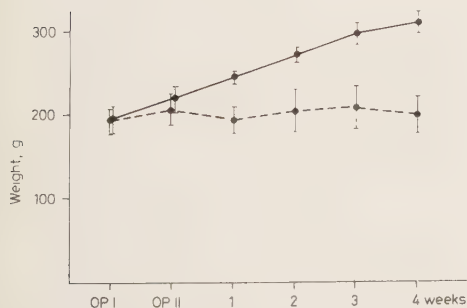


Fig. 2. Weight gain for uremic (-----) and sham-operated rats (———).

Table I. Some laboratory data in uremic and sham-operated rats (mean $\pm$ SD)

	Uremic rats	Sham-operated
Serum-creatinine, $\mu\text{mol/l}$	368 ± 80.3 (19)	51.6 ± 8.76 (9)
Creatinine clearance, $\mu\text{l/min/100 g b.w.}$	65.1 ± 25.9 (19)	688 ± 165 (9)
Serum urea, mmol/l	69.9 ± 13.4 (12)	7.46 ± 1.79 (5)
Serum sodium, mmol/l	148 ± 9.57 (12)	142 ± 3.42 (7)
Serum potassium, mmol/l	5.44 ± 0.79 (12)	4.80 ± 0.31 (7)
Serum chloride, mmol/l	106 ± 9.80 (12)	104 ± 4.63 (7)
Serum calcium, mmol/l	1.86 ± 0.37 (12)	2.37 ± 0.15 (7)
Serum phosphate, mmol/l	3.98 ± 0.80 (12)	2.70 ± 0.23 (7)
Serum magnesium, mmol/l	1.32 ± 0.27 (12)	1.24 ± 0.18 (5)
Plasma bicarbonate, mmol/l	19.0 ± 1.94 (9)	20.8 ± 0.45 (5)
Hematocrit, %	32 ± 1.69 (8)	48.4 ± 1.52 (5)

Number of rats in parentheses.

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Small Intestinal Dipeptidases and Disaccharidases in Experimental Uremia in Rats

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Key Words. Uremia · Chronic renal failure · Intestinal dipeptidases · Intestinal disaccharidases

Abstract. Rats were made severely uremic with partial nephrectomy (24-hour creatinine clearance 10% of normal). Jejunal dipeptidase activities (substrates: glycyl-*L*-leucine, *L*-alanyl-*L*-proline, and *L*-methionyl-*L*-methionine), disaccharidase activities (maltase, sucrase, trehalase, and lactase) and morphology were studied. A highly significant increase in glycyl-*L*-leucine and *L*-methionyl-*L*-methionine dipeptidases was found in uremic rats compared with controls. Proline dipeptidase activities were unaltered. Disaccharidase activities showed a slight increase in sucrase in uremic rats; otherwise no change was found.

Symptoms from the gastrointestinal tract – such as nausea, vomiting, diarrhea, and abdominal pain – occur in chronic renal failure (CRF). In the terminal stage, hemorrhagic and ulcerative mucosal lesions are often found in the gastrointestinal tract [16]. Recently, morphological [1, 5, 18, 24] and enzymatical [5, 15, 18] abnormalities were demonstrated in the small intestinal mucosa in patients with CRF. These findings suggest that in this condition a small intestinal dysfunction exists.

Experimental uremia offers a possibility to investigate its influence on the small intestinal function under standardized conditions. This report describes an investigation of the morphology of the mucosa, intestinal dipeptidase activities (glycyl-*L*-leucine dipeptidase EC 3.4.13.2, proline dipeptidase EC 3.4.13.9, and *L*-methionyl-*L*-methionine dipeptidase activity), and disaccharidase activities (maltase 3.2.1.20, sucrase 3.2.1.26, trehalase 3.2.1.28, and lactase 3.2.1.23) in uremic rats.

Materials and Methods

Male rats (Wistar strain, Møllegaard, Copenhagen, Denmark) were used, weight 150–200 g, with free access to commercial laboratory

animal diet (Ewos, Astra, Sweden) with a protein content of 24% and water *ad libitum*.

Experimental Design and Controls

Uremia was induced by a modification of the five-sixths nephrectomy method [27]. Resection and irradiation of left kidney parenchyma was followed 1 week later by contralateral nephrectomy. The abdominal organs were protected by a lead shield during irradiation of the left kidney. 4 weeks after the second operation, the rats were placed in metabolic cages, and urine was collected for determination of 24-hour creatinine clearance. They were then fasted for 12 h and anesthetized with ether. Upper jejunum was removed and a piece approximately 35 cm from pylorus was fixed in 4% formal solution for morphological examination. The next proximal 5 cm was used for enzyme analyses either directly or stored enveloped airtight in parafilm at -20°C until analysis. Blood samples were collected by cardiac puncture.

Sham-operated and irradiated rats, and also nonoperated, non-irradiated rats served as controls.

Enzyme Assays

Intestinal and liver dipeptidases (substrates: glycyl-*L*-leucine, *L*-alanyl-*L*-proline, *L*-methionyl-*L*-methionine from Sigma, St. Louis, Mo.) were determined according to *Josefsson and Lindberg* [10] under optimum pH condition [11]. Tris HCl, 50 mmol/l, pH 8.4, was used for methionyl-*L*-methionine. Intestinal disaccharidases were determined according to *Dahlqvist* [4].

One unit of enzyme activity hydrolyses 1 μmol substrate/min. Dipeptidase activity was expressed in U/mg nitrogen (16% of the protein content) and disaccharidase activity in U/g protein. Protein was

Table I. Some laboratory data in experimental uremia in rat (mean $\pm$ SD)

	Uremic rats		Sham-operated rats	
	values	n	values	n
Serum creatinine, $\mu\text{mol/l}$	363.5 $\pm$ 87.7	28	49.7 $\pm$ 7.77	18
Creatinine clearance, $\mu\text{l/min/100 g BW}$	66.5 $\pm$ 25.9	28	637.2 $\pm$ 175.7	18
Serum urea, mmol/l	69.9 $\pm$ 13.4	12	7.46 $\pm$ 1.79	5
Serum sodium, mmol/l	148.0 $\pm$ 9.57	12	142.0 $\pm$ 3.42	7
Serum potassium, mmol/l	5.44 $\pm$ 0.79	12	4.80 $\pm$ 0.31	7
Serum chloride, mmol/l	105.6 $\pm$ 9.80	12	104.1 $\pm$ 4.63	7
Serum calcium, mmol/l	1.86 $\pm$ 0.37	12	2.37 $\pm$ 0.15	7
Serum phosphate, mmol/l	3.98 $\pm$ 0.80	12	2.70 $\pm$ 0.23	7
Serum magnesium, mmol/l	1.32 $\pm$ 0.27	12	1.24 $\pm$ 0.18	5
Plasma bicarbonate, mmol/l	19.0 $\pm$ 1.94	9	20.8 $\pm$ 0.45	5
Hematocrit, %	32.0 $\pm$ 1.69	8	48.4 $\pm$ 1.52	5
Weight gain, g	10.4		125.2	

determined according to Lowry *et al.* [14] with bovine albumin as standard.

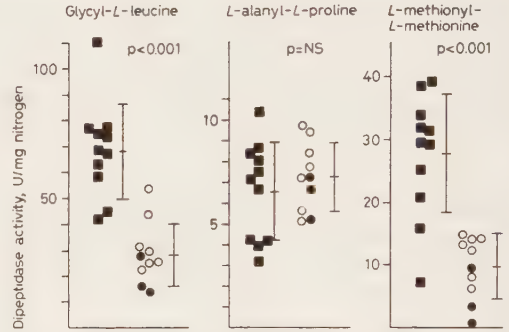
Laboratory Tests and Statistics

Serum creatinine, serum-urea, serum electrolytes, and hematocrit were determined by routine methods (Medilab Stockholm and Department of Clinical Chemistry, General Hospital, Malmö, Sweden).

The significance of differences was calculated by Student's *t* test.

Results

The rats developed pronounced uremia; they did not gain weight; after 4 weeks, serum creatinine was 7 times higher than that in the controls; and 24-hour endogenous creatinine clearance was about 10% of that in the controls. Table I lists the results of the laboratory tests in the controls and in the uremic rats. The histological examination of the small intestinal mucosa in the uremic rats showed no abnormalities. (The histological examination was kindly performed by Prof. N.O. Berg, Department of Pathology, University of Lund.)

**Fig. 1.** Intestinal dipeptidase activities (mean $\pm$ SD) in uremic rat (■), sham-operated and irradiated rat (●) and control rat (○).

Control experiments showed that the dipeptidase activities decreased when the mucosa was stored at -20°C for more than a week. The reduction was greater in uremic than in control rats, and greater for *L*-methionyl-*L*-methionine than for glycyl-*L*-leucine dipeptidase activities. Storage did not affect proline dipeptidase. Therefore the dipeptidase activities were analysed immediately; figure 1 shows the results. Both glycyl-*L*-leucine and *L*-methionyl-*L*-methionine dipeptidase activities were higher in uremic than in control rats ($p < 0.001$); proline dipeptidase activities were unchanged. Measurement of liver dipeptidases revealed no change in uremic animals compared with controls (fig. 2).

There was a slight increase in sucrase activity ($p < 0.01$) in uremic rats, otherwise no difference in disaccharidase activities was found between the groups (fig. 3).

Discussion

Experimental methods for inducing uremia in animals vary. With the most commonly used 5/6 nephrectomy method [3, 6, 21, 23], the rats become only slightly or moderately uremic due to hypertrophy of the remaining renal parenchyma. Mehls *et al.* [19] could reduce the creatinine clearance to 20% of that of controls by combining 7/8 nephrectomy with local irradiation. We produced a more profound uremia – creatinine clearance of 10% – by extending the resection of the kidney. The rats had hyperkalemia, hypocalcemia, hyperphosphatemia, anemia, and slight acidosis. All these abnormalities compare well with those found in patients with CRF.

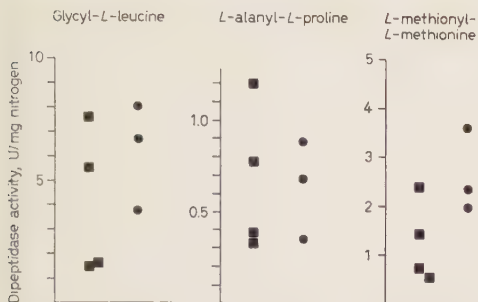


Fig. 2. Liver dipeptidase activities in uremic rat (■), sham-operated and irradiated rat (●).

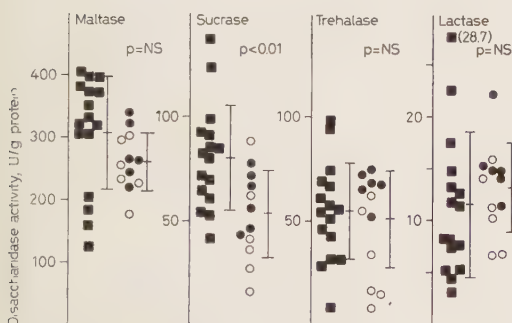


Fig. 3. Intestinal disaccharidase activities (mean $\pm$ SD) in uremic rat (■), sham-operated and irradiated rat (●) and control rat (○).

In patients with CRF the following abnormalities have been found in the small intestinal mucosa: morphological changes, e.g., shortening of villi, elongation of crypts, infiltration of inflammatory cells [1, 5, 18, 24], decreased glycyl-leucine, and proline dipeptidase activities [5]; decreased disaccharidase activities [5, 15, 18]. The intestinal dipeptidases studied are located to the cytoplasm of the enterocytes [22] and the disaccharidases to the brush border region [20]. Thus both groups of enzymes are affected in CRF in man.

In the uremic rats, we could not reproduce any sign of morphological changes. Our results on intestinal dipeptidases agree with those of *Wizemann et al.* [30]. They found increased *L*-methionyl-*L*-methionine, *L*-methionyl-*L*-leucine, *L*-leucyl-glycine, and glycyl-*L*-leucine splitting activ-

ities in the intestinal brush border of the chronic uremic rat. *Grimmel et al.* [7, 8] observed an increase of *L*-leucyl-*L*-proline and *L*-methionyl-*L*-proline in whole homogenate of uremic rat mucosa. These substrates and *L*-alanyl-*L*-proline, such as we used, are split by the same enzyme [25], and we did not observe any change. *Grimmel et al.* [7, 8] also showed a decrease of sucrase and maltase activities, whereas *Wizemann et al.* [30] found an increase of maltase and no change in lactase and sucrase. We found no change in disaccharidase activities apart from a slight increase of sucrase. The cause of these dissimilar results is obscure. One possible explanation is differences in length and severity of the experimental uremia. In all studies, the rats were maintained on an unrestricted diet.

The cause of the discrepant findings in uremic rat and in uremic man is difficult to explain. In this context, it is of interest to note that uremic man has an increased number of mitoses in the crypts [1], whereas the contrary is true in the uremic mouse [2, 17]. An explanation for these discrepancies between man and animal, besides species differences, might be a different intake of energy and nutrients, e.g., protein. Our rats were given an unrestricted diet, whereas in man, protein restriction is maintained. Starvation and protein deprivation influence cell renewal [9, 29] and decrease dipeptidase activities in rat [12, 26] and in man [13]. As discussed above, the length of the uremic period might also play a role in the development of the gastrointestinal dysfunction in CRF.

The increased intestinal dipeptidase activities observed could perhaps be part of a generalized increased dipeptide splitting activity due to uremia. Both decreased and increased liver enzyme levels related to amino acid and urea metabolism have been found in chronic uremic rats [28]. Our measurements of liver dipeptidases, however, show that increased glycyl-*L*-leucine and *L*-methionyl-*L*-methionine dipeptidase activities do not seem to be a generalized phenomenon in uremia. The influence that the dietary intake of nutrients—especially proteins—has on these enzymes is not known. Further studies on rats with a regulated dietary intake of, e.g., protein, will probably elucidate this matter.

Acknowledgments

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III

SMALL INTESTINAL DIPEPTIDASES AND DISACCHARIDASES IN UREMIC RATS ON A LOW PROTEIN NUTRITION

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Abstract

Small intestinal dipeptidases (substrate: glycyl-L-leucine, L-methionyl-L-methionine, and L-alanyl-L-proline) were determined in uremic and sham-operated rats given normal protein diet (24%), low protein diet (6%), and total parenteral nutrition for 10 to 14 days. Disaccharidases (substrate: maltose, sucrose, trehalose, and lactose) were measured after normal and low protein diet orally.

Glycyl-L-leucine and L-methionyl-L-methionine splitting activities were increased on normal protein diet but decreased after low protein feeding in uremic rats. Parenteral nutrition further lowered the enzyme activity. L-alanyl-L-proline dipeptidase activity showed an inverse relation to protein intake with the highest values after parenteral nutrition. Lactase increased in uremic rats after low protein feeding and was the only disaccharidase to be affected by the nutritional change. It is suggested that the changes of the intestinal dipeptidase activities in uremia are adaptive to variation in the luminal content of di- and oligopeptides because of the type of nutrition given.

Introduction

We recently reported that severely uremic rats showed significant increase in the activity of two intestinal dipeptidases (1). In patients with chronic renal failure, the activities of some small intestinal dipeptidases and disaccharidases were decreased (2). The reason for this discrepancy is unknown. One difference which may have played a role is that the rats were fed a diet with a normal protein content, whereas the patients' intake of protein were reduced.

This study elucidated the effect of a reduction in dietary protein and also the effect of total parenteral nutrition (TPN) on the activity of glycyl-L-leucine dipeptidase (EC 3.4.13.2), proline dipeptidase (EC 3.4.13.9), and L-methionyl-L-methionine dipeptidase in the intestinal mucosa of severely uremic rats. Disaccharide activities (maltase 3.2.1.20, sucrase 3.2.1.26, trehalase 3.2.1.28, and lactase 3.2.1.23) were also measured after normal and low protein nutrition orally.

Methods

Male Wistar rats (Møllegaard, Copenhagen, Denmark) weighing 150 - 200 g were used. Uremia was induced by partial nephrectomy and irradiation of the left kidney followed by contralateral nephrectomy one week later, as earlier described (3). Sham-operated rats, where the left kidney had been irradiated, served as controls.

Dipeptide splitting activities were determined according to Josefsson and Lindberg (4) under optimum pH condition (5). Tris-HCl, 50 mmol/l, pH 8.4 was used for L-methionyl-methionine. Dipeptidase activities were expressed in U/mg nitrogen (16% of the protein content). Intestinal disaccharidases were determined according to Dahlqvist (6) and given as U/g protein. Lactase was also measured with p-chloromercuribenzoic acid (PCMB), which has been shown to inhibit the lysosomal acid β -galactosidase and thus specifically assay brush border lactase activity (7). Protein was assessed according to Lowry et al. (8) with bovine albumin as standard.

Uremic and sham-operated rats were fed an ordinary laboratory animal diet (R3, Astra-Ewos, Sweden) ad libitum during the first 1 to 2 weeks. They were then given various diets or TPN as follows:

Normal protein diet: Altogether 24 uremic rats and 19 sham-operated rats continued for 4 weeks after the second operation on the laboratory animal diet. This was given ad libitum and contained 24% protein as soya meal and crude fish protein.

Low protein diet: 10 uremic and 10 sham-operated rats were fed a 6% egg protein diet ad libitum. The low protein nutrition was instituted 14 days after the last operation and fed for 14 days.

All rats were kept in metabolic cages to accurately measure food intake. Table I shows the ad libitum intake of the oral diets in uremic and sham-operated rats.

After the feeding periods the rats were fasted overnight, then anaesthetized with ether; a part of the jejunum, approximately 30 cm from pylorus, was removed for enzyme analysis. Blood was collected by cardiac puncture.

Total parenteral nutrition: This part of the study was performed at Vitrum institute for Human Nutrition, Stockholm, Sweden. In connection with the contralateral nephrectomy, a permanent cathe-

TABLE I. Ad libitum daily intake of two different diets in uremic and sham-operated rats.

	24% protein diet ^a		6% protein diet ^b	
	uremic (n=8)	controls (n=8)	uremic (n=10)	controls (n=10)
Food intake, g/kg b.w.	74.3	74.8	79.1	83.6
Nitrogen, g/kg b.w.	2.9	2.9	0.59	0.62
Carbohydrate, g/kg b.w.	36.1	36.4	63.8	67.4
Fat, g/kg b.w.	4.0	4.0	4.8	5.0
Energy, kJ/kg b.w.	1190	1200	1270	1340

a/ 24% protein diet (g/kg diet): fish protein, soya meal (241); cereals, wheat germs, wheat middlings (486); animal and vegetable fat (54); fibre (39); minerals and vitamins (63). Energy 16 kJ/g diet.

b/ 6% protein diet (g/kg diet): egg albumin powder (60); wheat starch (269); sucrose (269); glucose (269); soybean oil (60); fibre (10); minerals and vitamins (63). Energy 16 kJ/g diet.

TABLE II. Composition of total parenteral nutrition given to uremic and sham-operated rats. CAA = complete mixture of essential and nonessential amino acids, EAA = essential amino acids + histidine.

Component	Given amounts g/kg body weight/day	Solution
Nitrogen	0.6	CAA (Vamin [®]) EAA (Aminess [®])
Carbohydrate	61-63	Glucose 50%
Triglycerides	9	Intralipid [®] 20%
Egg yolk phospholipids	0.55	
Glycerol	1.0	
Total energy supply: 1385 kJ/kg body weight/day		
Total volume supply: 280 ml/kg body weight/day		

ter was inserted into vena cava superior via vena jugularis externa on 6 uremic and 5 sham-operated rats, as described by Roos et al. (9). TPN was given from day 10 to 20 after this operation and included fat (Intralipid[®] 20%), 50% glucose, and either essential amino acids, EAA (Aminess[®]), or a complete mixture of essential and nonessential amino acids, CAA (Vamin[®]). TPN was given at low nitrogen level (0.6 g N/kg body weight/day) (the composition is shown in Table II). The energy supply was 1385 kJ/kg body weight/day. Vitamin and mineral additive solutions were used to provide a complete nutrition adapted to the requirement of the rat (Wennberg et al., to be published). After completion of the parenteral nutrition, animals were anaesthetized with sodium pentobarbitone intraperitoneally and a portion of jejunum excised as above. Blood

was collected by cardiac puncture. The intestine was kept frozen at -15 to -20° C until enzyme analysis was performed 1 to 4 days later.

Dipeptidases were analysed in all groups of rats, putting the rats, given TPN with EAA and CAA, together into one group. Disaccharidases were determined only after the two oral diets.

Laboratory analysis and statistics: Serum creatinine was analysed on a Prisma multichannel analyser using a kinetic Jaffé-reaction.

The statistical significance of differences were evaluated with analysis of variance (one-way classification, fixed effects) for data shown in Table III (10). Otherwise Student's t-test was used.

Results

Uremic rats demonstrated significantly higher serum creatinine ($p < 0.001$) than sham-operated rats in all dietary groups; 345 ± 102 $\mu\text{mol/l}$ (mean $\pm$ SD) versus 52 ± 11 for 24% protein diet, 221 ± 69 versus 75 ± 13 for 6% diet, and 193 ± 69 versus 56 ± 7.9 for TPN. Uremic animals given the low protein diet and TPN had a lower serum creatinine ($p < 0.01$) compared to uremic rats on the normal protein containing diet.

The intake of the diets were reduced in uremic rats; but estimated per kg of body weight, there was no difference compared to non-uremic rats (Table I). Uremic rats on 6% protein diet consumed each day about 20% of the nitrogen intake of the uremic rats on 24% diet, whereas carbohydrate consumption was nearly twice as high.

Fig. 1 shows the weight curves before and during the different nutritional regimens. The 6% protein diet resulted in temporary growth retardation in sham-operated rats and a constant weight level in uremic rats. Uremic rats on the 24% protein diet similarly failed to gain weight. TPN resulted in weight increase for both groups of rats, comparable to that of non-uremic rats on 24% protein diet.

The glycyl-L-leucine and L-methionyl-L-methionine dipeptidase

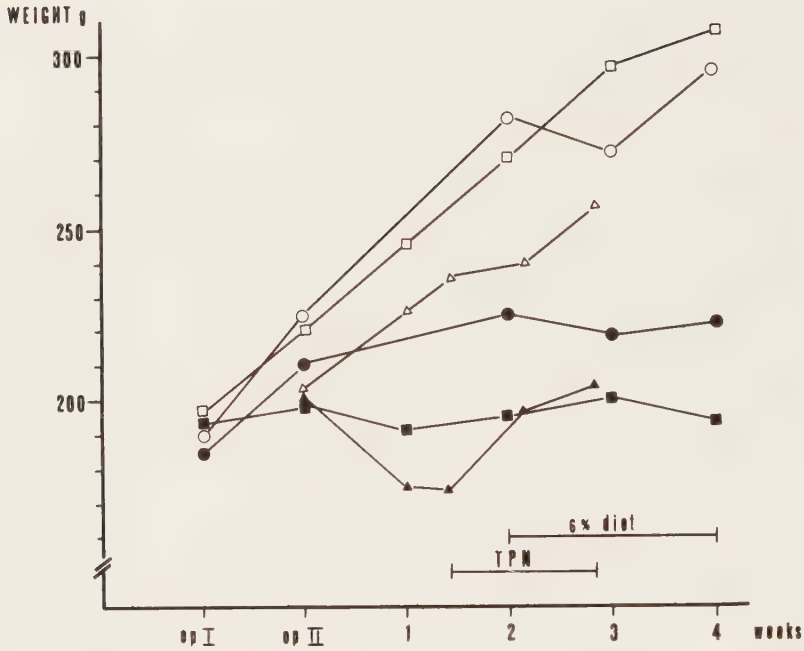


Fig. 1. Weight curves (mean values) for uremic (filled symbols) and sham-operated rats (open symbols). $\square$ = 24% protein diet, $\circ$ = 6% protein diet, Δ = total parenteral nutrition.

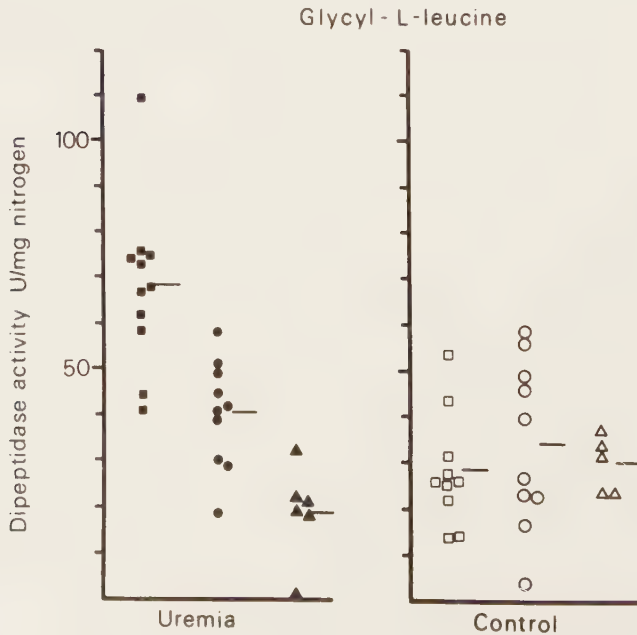


Fig. 2a

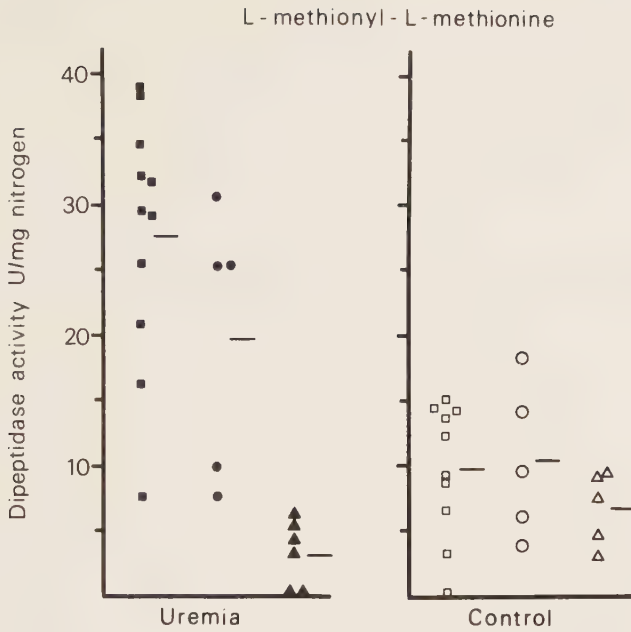


Fig. 2b

Fig. 2a-c. Small intestinal dipeptidases in rats given normal protein diet ($\square$), low protein diet ($\circ$), and total parenteral nutrition (Δ). Filled symbols represent uremic animals and open symbols sham-operated animals. Mean value is given for each group.

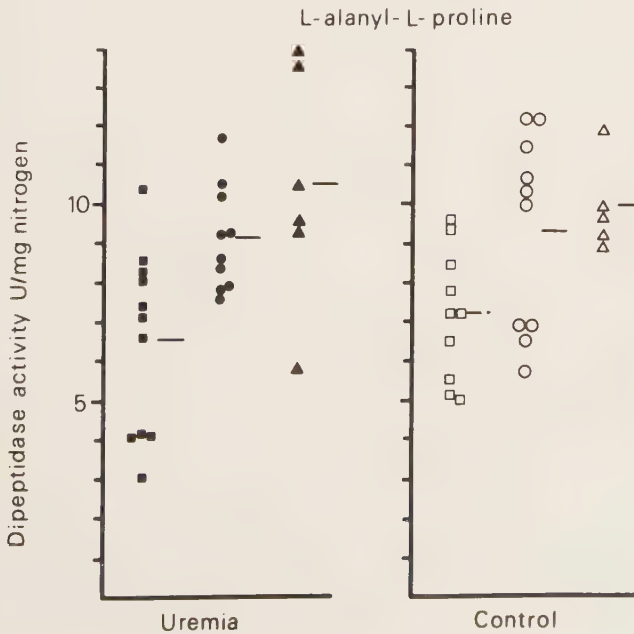


Fig. 2c

TABLE III. Mean dipeptidase activities (U/mg N) in uremic and sham-operated rats and F-ratio from analysis of variance.

	Diet		TPN	Statistics	
	24% protein	6% protein		F	p
Relative oral protein intake	3.5	1	0		
Glycyl-L-leucine dipeptidase					
uremic	68.2	40.5	19.1	23.4 F(2/24)	<0.001
controls	28.4	34.1	30.3	<1	N.S.
L-methionyl- L-methionine dipeptidase					
uremic	27.8	19.8	3.22	16.2 F(2/19)	<0.001
controls	9.84	10.5	6.87	<1	N.S.
L-alanyl-L-proline dipeptidase					
uremic	6.54	9.12	10.5	6.93 F(2/24)	<0.01
controls	7.22	9.29	9.94	4.11 F(2/22)	<0.05

activities (Fig. 2a-b and Table III), which were increased in uremic rats on 24% protein feeding, decreased after low protein oral feeding and were further lowered after total parenteral nutrition ($p < 0.001$). In non-uremic rats there was no significant difference between the three dietary groups for either of these two enzyme activities. Concerning proline dipeptidase (Fig. 2c), a tendency was found towards higher enzyme activities when oral protein intake was reduced ($p < 0.01$ for uremic rats, $p < 0.05$ for sham-operated rats; Table III).

Figure 3 shows the values of disaccharidase activities. The increased sucrase level noted in the uremic rats compared with sham-operated rats on 24% protein diet ($p < 0.01$) remained after 6% protein diet ($p < 0.01$). Lactase both with and without PCMB was significantly increased in uremic rats fed 6% protein diet compared with 24% protein diet ($p < 0.05$). A similar tendency was noted for sham-operated rats, although not statistically significant. The activity of brush border lactase in the different groups constituted 91 to 96% of total lactase activity. Maltase and trehalase were not affected by the nutritional alterations.

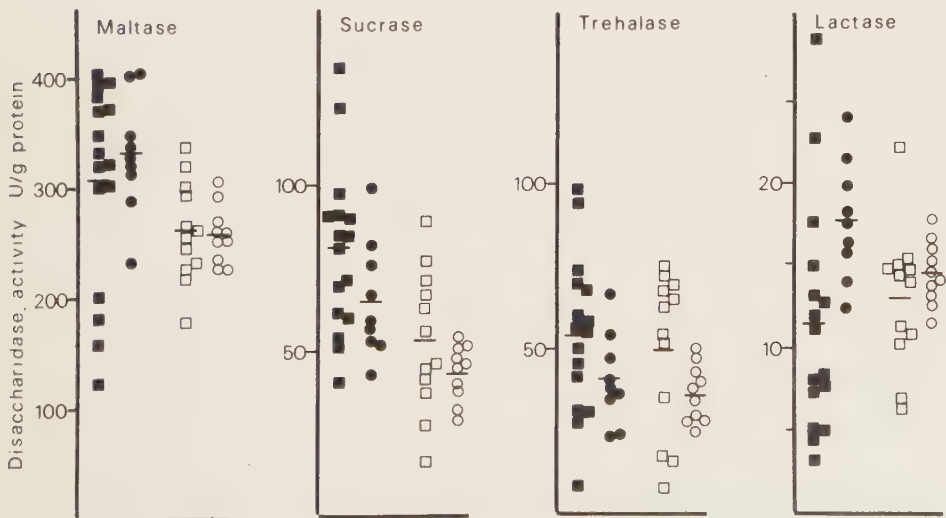


Fig. 3. Small intestinal disaccharidases in uremic and sham operated rats given 24% protein diet and 6% protein diet. Symbols as in Fig. 2. Mean value.

Discussion

No previous data has to our knowledge been published concerning the effects of dietary protein on intestinal enzymes in uremia. However, experimental studies on non-uremic animals are available. Deprivation of oral protein to animals without uremia has been shown to effect small intestinal peptidases (11, 12, 13, 14). Kumar and Chase (12, 13) found reduced glycyl-proline and valyl-proline splitting activities in rat and monkey small intestinal mucosa after low protein feeding. Studying rats starved for 5 days Kim et al. (15) found brush border enzyme activities to be decreased, whereas dipeptidases in the cytosol were increased. Nicholson et al. (14) observed lower brush border peptide hydrolase activities in the small intestine from rats fed a low protein compared to a high protein diet. They also tested four cytosol dipeptidases and found three to be lowered after the low protein diet. Thus both brush border and cytosol peptide hydrolase activities have been found to be decreased in animals when protein intake was lowered. Studies in protein malnourished patients have given comparable results (16, 17, 18).

Uremic rats given a diet with normal protein content exhibit increased activities of some dipeptidases in homogenate of mucosa and in brush border preparations (1, 19, 20, 21). We found that glycyl-L-leucine and L-methionyl-L-methionine dipeptidase activities were increased, but that L-alanyl-L-proline dipeptidase activity was unchanged in uremic rats given a normal diet (24% protein) (1). Our results presented in this paper indicate that glycyl-L-leucine and L-methionyl-L-methionine dipeptidase activities decrease when low (6%) protein feeding is instituted, whereas L-alanyl-L-proline dipeptidase activity increases. After a period of parenteral nutrition when no food reached the intestine, these changes of enzyme activities were emphasized even further. Since the dipeptidase activity is expressed as units per mg nitrogen in the mucosa, a change in protein content in the mucosa could affect the relative enzyme levels. Calculation of the ratio between mg nitrogen and mg wet weight of the intestine gave slightly higher values in control rats on normal diet; otherwise, no difference between the groups on various dietary treatment was found. Thus the changed dipeptidase activities were not the result of altered nitrogen content in the mucosa.

Serum creatinine was lower among uremic rats given low protein or parenteral nutrition compared with normal diet. Further evaluation of kidney function was not made in these animals. However, we earlier found 24-hours creatinine clearance, a more reliable test of kidney function, to be similar in the three dietary groups (Wennberg et al., to be published).

Glycyl-L-leucine and L-alanyl-L-proline are split by two different enzymes, glycyl-L-leucine dipeptidase and proline dipeptidase (22, 23). Glycyl-L-leucine dipeptidase has a broad specificity and has been shown to hydrolyse also L-methionyl-L-methionine (24). Proline dipeptidase, on the other hand, is restricted mainly to aminoacyl-L-proline containing peptides (23). The reason for the different behaviour of the two enzymes in uremic rats fed normal or low protein diet is obscure. Glycyl-L-leucine and proline dipeptidase have both been localized mainly to the cytoplasm of the enterocyte (25). Besides, a minor fraction of the glycyl-L-leucine splitting activity can be ascribed to an aminopeptidase found in rat intestinal brush border (26, 27, 28). This enzyme does not hydrolyse aminoacyl-L-proline (27). Our dipeptidase preparation did not exclude brush border enzymes; their presence could have affected the total glycyl-L-leucine activity.

Of the brush border enzymes studied, sucrase was found to be increased in uremic rats given both 24% and 6% protein diets, whereas lactase was normal after 24% protein feeding but increased after low protein diet in uremic rats. The elevated lactase level is noteworthy considering that the 6% protein diet was lactose-free. Increased lactase activity has earlier been reported in poorly nourished and low protein fed rats (29, 12). Our results show that lactase in this way behaved like the cytosol enzyme proline peptidase, but their cellular distribution was different, as the increased lactase activity mainly originated from brush border.

It thus seems unlikely that the discrepancies between the dipeptidases and between the disaccharidases in uremic small intestine can be explained by different cellular localization of the enzymes.

A plausible explanation for the increase in glycyl-L-leucine dipeptidase activity in experimental uremia with normal protein

feeding is that there is a raised content of di- and oligopeptides in the intestinal lumen. Clinical studies have suggested that peptides with a molecular weight of approximately 500-1500 Dalton accumulate in uremia (30).

When protein load is reduced during low protein feeding, dipeptide and oligopeptide content decrease and may reach the level of non-uremic rats. The enzyme activity in these rats was comparable with that in non-uremic rats. During total parenteral nutrition the peptide content may decrease further and the enzyme activity in these rats was still lower. Thus the changes of the enzyme activity may be explained as an adaptive phenomenon. The observed discrepancies between glycyl-L-leucine dipeptidase and proline dipeptidase might be explained by the different specificities of the two enzymes, as discussed above.

The cause of the low small intestinal dipeptidase activities found earlier in uremic patients (2) is possibly the reduced protein intake. In accordance with this analyses of small intestinal biopsies from another two uremic patients, with an intake of approximately 40 g protein per day, showed low to normal glycyl-L-leucine dipeptidase activities (Sterner & Lindberg, unpublished observation).

In conclusion; variation in dietary protein content affects the intestinal dipeptidase activities, which must be taken into account when considering intestinal enzymes in uremia. It is of particular interest concerning dietary treatment in uremia, e.g., when changing from high to low protein diet, from protein to amino acid diet, and from parenteral to oral nutrition.

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IV

IN VIVO AND IN VITRO ABSORPTION OF AMINO ACIDS AND DIPEPTIDES IN SMALL INTESTINE OF UREMIC RATS

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Abstract

The small intestinal absorptive capacity in partially nephrectomized rats was studied in vitro and in vivo. Severe uremia reduced the in vivo uptake of glycine, L-methionine, and L-lysine, while the dipeptides glycyl-L-valine, glycyl-L-leucine, and glycyl-L-lysine were not similarly affected. The in vitro study using everted rings failed to show any changes in the absorption of amino acids, dipeptides, and glucose in uremic rats. The results indicate that in uremia as in other adverse conditions, the amino acid absorptive step is more severely damaged than that of the dipeptide.

Introduction

Essential amino acids given orally form an important part of the dietary treatment in chronic renal failure (CRF) (7). However, we do not know anything about the capacity of the uremic small intestine to absorb amino acids and their corresponding dipeptides. It seems plausible to presume that the absorptive function of the uremic mucosa is disturbed because morphological and enzymatical abnormalities have been found in the small intestinal mucosa in man with CRF (2,6,15,16) and in uremic rat (8,9,20,21). This paper describes the in vitro absorption in uremic rats of glycine, leucine, glycyl-leucine, and glucose. Furthermore, the in vivo absorption of glycine, valine, lysine, leucine, methionine, glycyl-valine, glycyl-leucine, glycyl-lysine, and alanyl-proline was studied.

Material and methods

Male rats (Wistar strain, Møllegaard, Copenhagen, Denmark) weighing 150-200 g were made uremic by partial nephrectomy as described earlier (19). Control rats were sham-operated. The animals had free access to commercial laboratory diet (R3, Astra-Ewos, Sweden) with a protein content of 24% and water ad libitum. The experiments were performed after 4 weeks with the animals fasted overnight in metabolic cages with free access to water.

The amino acids, dipeptides, and polyethylene glycol 4000 (PEG) were purchased from Sigma, St. Louis, USA, and D-glucose from Kebo, Stockholm, Sweden. The concentration of the amino acids was chosen to correspond to their normal concentration in intestinal content during digestion in the rat (18).

In vitro absorption

With the rats under ether anaesthesia 15 cm of the upper jejunum, beginning 15 cm from pylorus, was removed. The jejunal segment was everted over a steel rod and cut into 2-3 mm rings (1). Care was taken to avoid Peyers patches. The rings were immediately placed in Krebs-Ringer phosphate buffer containing half the standard concentration of calcium (4,5).

The amino acids, dipeptide, or glucose were dissolved in the Krebs-Ringer phosphate buffer (see above), gassed with 100% O₂, and the corresponding isotope labelled compounds (Radiochemical Centre, Amersham, England), were added to give a radioactivity of 0.2 μ Ci per ml incubation solution. These were 2.5 mmol/l (1-¹⁴C) glycine + 2.5 mmol/l L-leucine; 2.5 mmol/l glycine + 2.5 mmol/l L-(U-¹⁴C) leucine; 2.5 mmol/l (1-¹⁴C) glycyl-L-leucine; 2.5 mmol/l glycyl-L-(U-¹⁴C) leucine; 5 mmol/l D-(U-¹⁴C) glucose. One everted ring was incubated in 2 ml of incubation solution at 37° C under 100% O₂ for 5 minutes. The absorption of each substrate was measured in triplicate in each rat. After incubation, the ring was rapidly washed with physiological saline and gently blotted on hard filter paper. The ring was weighted and then homogenized with a manual glass homogenizer in 1 ml of redistilled water. The homogenate was boiled for 5 minutes and centrifuged (12 000 x g) for 15 minutes. 0.25 ml of the supernatant was mixed with 10 ml of 1.4-dioxan-based scintillation solution. The radioactivity was determined in a Packard-Tri-Carb model 33 75 liquid scintillation spectrometer. The content of protein in the solution did not cause quenching.

The uptake of various substances in the everted rings was expressed as the ratio between the radioactivity (cpm/ml) in the intracellular tissue fluid and in the incubation medium. We found total tissue fluid to be about 80% of tissue wet weight. Extracellular fluid, measured as inulin space (17), was in an earlier experiment found to be 11% of total tissue fluid with no difference between uremic and control rats.

In vivo absorption

Under ether anaesthesia the abdomen was incised and 2 jejunal segments (about 10-12 cm long, starting 10 cm from the pylorus) were isolated. Polyethylene tubings were inserted and secured by ligatures at both ends. Throughout the procedure, care was taken not to interfere with blood flow. The intestinal loops were washed with isotonic saline and emptied by air from a syringe. The solutions used contained either 3 mmol/l glycine + 3 mmol/l L-leucine + 3 mmol/l L-valine + 3 mmol/l L-lysine + 6 mmol/l L-methionine or 3 mmol/l glycyl-L-leucine + 3 mmol/l glycyl-L-valine + 3 mmol/l glycyl-L-lysine + 3 mmol/l L-methionyl-L-methionine. 0.4% PEG (4000) was used as a non-absorbable marker, making it possible to correct for water transport across the mucosa. pH was adjusted to 6.8 using 0.2 mol/l Tris-HCl. The proximal of the two intestinal segments was filled with 1.5 ml of either of the solutions. After 5 minutes the loop was emptied and the fluid collected in a plastic test-tube. The distal segment was then similarly tested. Every second time the amino acid solution was placed in the proximal loop and the dipeptides in the distal loop and every other time vice versa. After absorption the solutions were deproteinized with 50 mg sulfosalicylic acid per ml and stored at -70°C until analysed.

In a separate experiment a solution containing 3 mmol/l glycyl-L-leucine and 3 mmol/l L-alanyl-L-proline was tested on 5 uremic and 5 sham-operated rats. This solution was used in both the proximal and the distal loops on these rats.

Quantitative analysis of amino acids and dipeptides was carried out on an amino acid analyser (Kontron liquimat III). PEG was measured by the turbidimetric method according to Boulter & McMichael (3). Uptake of amino acids and dipeptides was calculated from the difference between the concentration in the solution before and after the absorption experiment. Correction was made for water transport according to the final PEG concentration.

Laboratory tests. Blood was collected by cardiac puncture. Serum creatinine was analysed at the Department of Clinical Chemistry, General Hospital, Malmö, Sweden.

Statistical evaluation. Differences between groups were calculated with the non-parametric Mann-Whitney's test.

Results

All partially nephrectomized rats developed considerable uremia with a median serum creatinine of 292 $\mu\text{mol/l}$ (range 184-551) for the group as a whole compared with 73 $\mu\text{mol/l}$ (range 37-99) for the sham-operated rats. The uremic animals gained very little or nothing in weight during the 4 weeks from the last operation to the experiments.

Figure 1 shows the results of the in vitro absorption of glycine, leucine, glycyl-L-leucine, and glucose. There was no significant difference in the uptake of the amino acids and the dipeptide between the uremic and sham-operated rats. Generally, leucine was absorbed faster than glycine and glycine uptake was more rapid from the dipeptide than from the equivalent amino acid mixture. Glucose was absorbed as fast in uremic rats as in the controls.

Figure 2 demonstrates absorption in vivo of amino acids. There is a reduced absorptive capacity with increasing uremia for glycine, lysine, and methionine ($p < 0.05$). The same tendency was noted for valine and leucine though the differences were not significant. The data for the dipeptide absorption (Fig. 3) show that there is no significant reduction for any of the corresponding dipeptides. L-methionyl-L-methionine was found to be unstable and was recovered mainly as methionine in the intestinal fluid after the tests. No estimation of the absorption of this dipeptide was therefore done. Using only physiological saline less than 0.1 mmol/l of amino acids was found in the intestinal content of uremic and control rats.

In the separate in vivo experiment absorption of L-alanyl-L-proline and glycyl-L-leucine in uremic rats was found to be 153 nmol/cm jejunum/5 minutes (range 84-290) and 155 nmol/cm jejunum/5 minutes (range 75-252). The corresponding values for sham operated animals were 195 (range 69-298) and 193 (range 36-303). There was no significant difference between uremic and sham-operated rats.

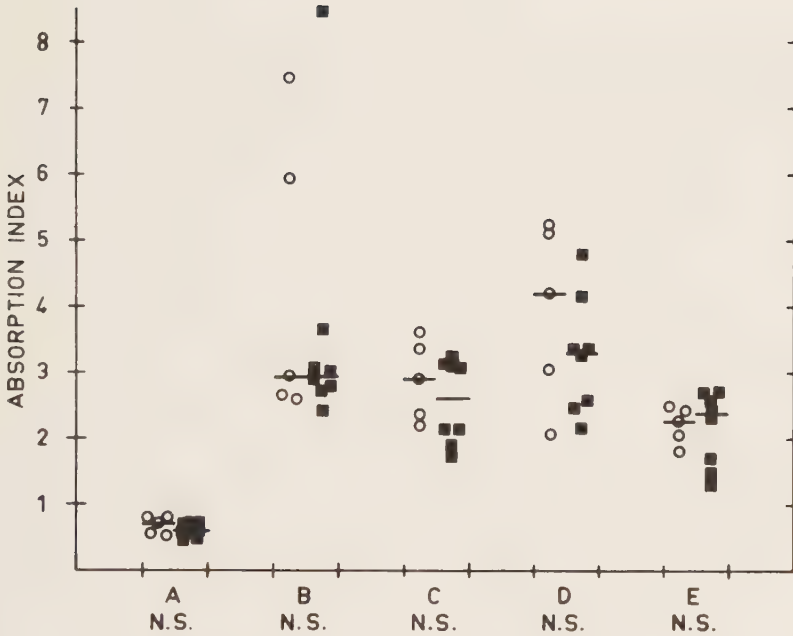


Fig. 1. In vitro absorption with everted jejunal rings in sham-operated (o) and uremic rats (■).

Median value.

A: ^{14}C -glycine (2.5 mmol/l) + L-leucine (2.5 mmol/l)

B: Glycine (2.5 mmol/l) + L- ^{14}C -leucine (2.5 mmol/l)

C: ^{14}C -glycyl-L-leucine (2.5 mmol/l)

D: Glycyl-L- ^{14}C -leucine (2.5 mmol/l)

E: D- ^{14}C -glucose (5 mmol/l)

Absorption index = $\text{cpm} \times \text{ml}^{-1}$ intracellular tissue fluid / $\text{cpm} \times \text{ml}^{-1}$ medium.

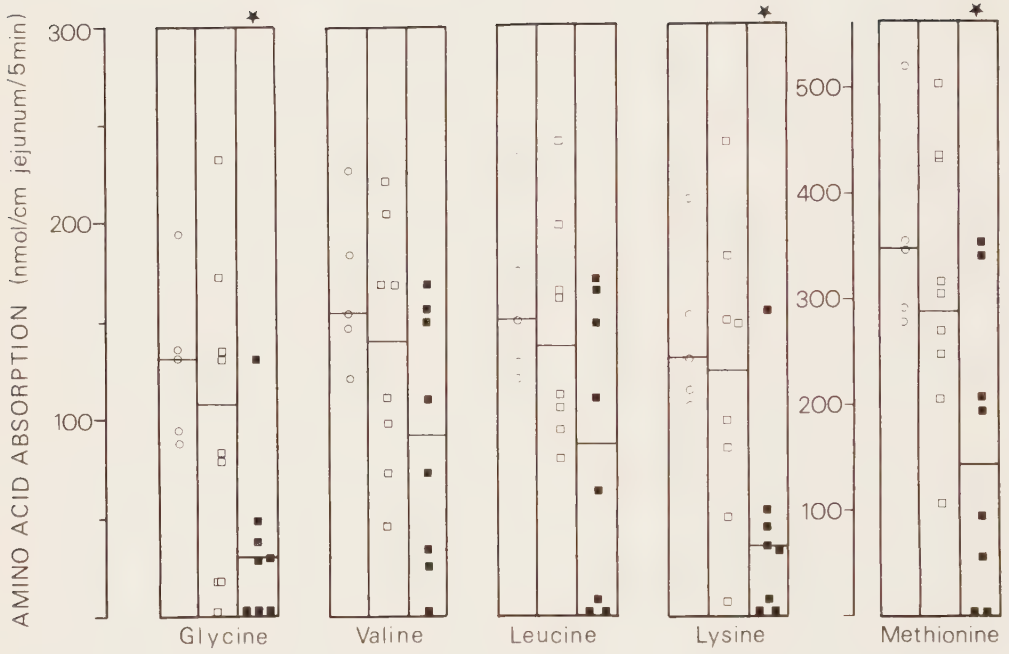


Fig. 2. In vivo absorption of glycine (3 mmol/l), valine (3 mmol/l), leucine (3 mmol/l), lysine (3 mmol/l), and methionine (6 mmol/l) in jejunum of sham-operated rats (○), uremic rats with serum creatinine less than 250 $\mu\text{mol/l}$ (□) and uremic rats with serum creatinine more than 250 $\mu\text{mol/l}$ (■).

Median value.

* = $p < 0.05$ compared with sham-operated rats.

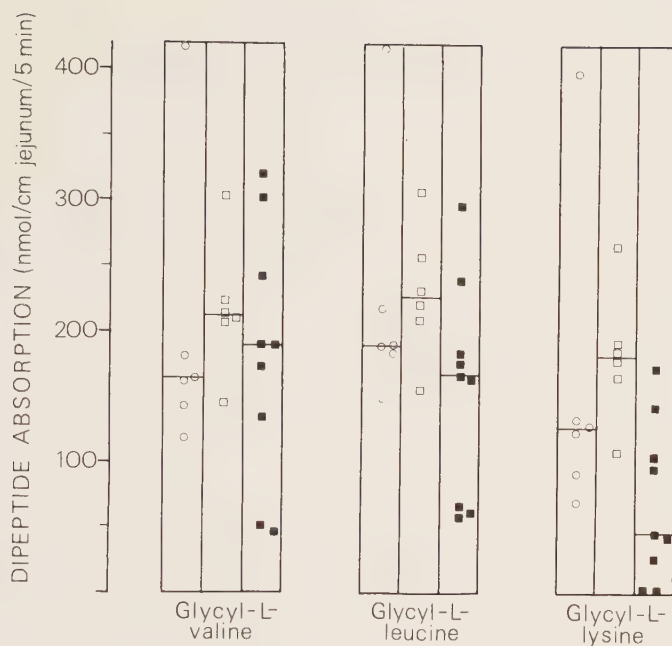


Fig. 3. *In vivo* absorption of glycyl-L-valine (3 mmol/l), glycyl-L-leucine (3 mmol/l) and glycyl-L-lysine (3 mmol/l) in jejunum of sham-operated and uremic rats. Symbols as in figure 2. Median value.

Discussion

Few intestinal absorption studies have previously been performed in uremia. Wizemann et al. (21) reported L-leucine absorption to be decreased in mild and increased in severe uremia in rat. In man, Bloch et al. (2) using intestinal perfusion technique found glucose absorption to be at the lower limit of normal in one patient. Denneberg et al. (6) found a lower rise of blood glucose at oral tolerance test in patients with CRF compared with controls.

Our results from *in vivo* experiments show that the absorption of some amino acids is reduced in severely uremic rats. This is in contrast to the findings of Wizemann et al. (21), who perfused isolated ileal segments with ^{14}C -L-leucine. As we used jejunal segments in our study this could explain the difference, but comparison of the results is hampered as their report gave no further details. In our study the absorption of dipeptides was not affec-

ted by the uremic state. This discrepancy between the absorption of the amino acids and of the dipeptides agrees with the findings that the amino acid absorptive mechanism (probably at the luminal site) is more affected by adverse conditions than that of the dipeptide (e.g. dietary changes in rat, and certain disease in man; 11,12,14). It supports the hypothesis of different entering mechanisms into the enterocyte for amino acids and dipeptides at the luminal membrane (13).

In this context it is also of interest to note that we earlier found (20) glycyl-L-leucine dipeptidase (EC 3.4.13.2) to be increased in uremic rats, whereas proline dipeptidase (EC 3.4.13.9) was unchanged as compared to control rats. In order to study the physiological significance of this result, absorption of glycyl-L-leucine and L-alanyl-L-proline was studied in a separate experiment. There was no difference in the handling of these two dipeptides in uremic and sham-operated rats. Thus the difference between the two dipeptidase activities had no effect on the uptake of the dipeptides.

In contrast to the *in vivo* test we could not demonstrate any effect of the uremia on the *in vitro* absorption of glucose, glycine, leucine, and glycyl-L-leucine. Earlier findings (13) that amino acids are absorbed faster if administered as peptides instead of as free amino acids were confirmed. We also found that the tissue content of glycine was lower than that of leucine after incubation with glycyl-L-leucine. The phenomenon is probably due to hydrolysis of some fraction of the dipeptide in the medium, and absorption of glycine at a lower rate than leucine. Glycyl-L-leucine dipeptidase and proline dipeptidase have proved to be rapidly released (within minutes) from the mucosa into the medium in this *in vitro* experimental model (10). Therefore results from dipeptide absorption studies *in vitro* must be accepted with caution.

The cause of the discrepant results from the *in vivo* experiments is obscure. One explanation may be that the *in vitro* absorption tests were not performed in an uremic environment. Harmful substances (e.g. so called uremic toxins) might be quickly released from the mucosa into the incubation medium. To test this everted rings from 9 controls and 7 uremic rats (median serum creatinine 210 $\mu\text{mol/l}$) were incubated in normal and uremic rat

serum containing L- ^{14}C -leucine (Sterner, unpublished observation). No significant depression of leucine uptake was noted using the uremic serum.

In conclusion this study has shown that in experimental uremia the in vivo absorption of some amino acids is decreased. The uptake of the dipeptides is not affected to the same extent. As amino acids are used in the dietary treatment of patients with uremia it is important to know whether these findings are also valid in man. Studies to elucidate this are in progress.

Acknowledgements

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V

EFFECTS OF TOTAL PARENTERAL NUTRITION IN RATS
WITH EXPERIMENTAL CHRONIC RENAL FAILURE

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Abstract

The effects of total parenteral nutrition (TPN) in partially nephrectomized rats (n=17) and sham-operated controls (n=12) were evaluated and compared to the effect of low and high nitrogen oral diets (6% and 24% protein). TPN included fat (9 g/kg/day), high energy (1385 kJ/kg/day), and low nitrogen content (0.6 g N/kg/day), corresponding to 8% protein) either as essential amino acids (EAA) or as a mixture of essential and non-essential amino acids (CAA). The parenteral nutrition was administered intravenously via a permanent catheter continuously for 10 days. Most animals tolerated the treatment with no signs of overhydration or electrolyte imbalances. Uremic rats on TPN gained similarly in weight compared to control animals, whereas uremic rats given oral diets showed a lower weight increase. Both amino acid solutions promoted positive nitrogen balance and growth. Plasma-urea dropped during TPN and low protein oral feeding in uremic and control rats, but not in the high protein fed animals. Serum creatinine decreased with TPN but not with oral feeding in uremic rats. Albumin and hemoglobin levels were significantly reduced in all uremic rats irrespective of dietary treatment. The experimental model presented here could be useful for further studies on parenteral nutrition in uremia.

Introduction

Treatment with low nitrogen dietary regimens or parenteral nutrition in which essential amino acids are provided as the primary nitrogen source has been used for patients with chronic renal failure (CRF) (1-8). Metabolic studies of nitrogen utilization and amino acid metabolism are, however, often difficult to perform in these patients due to other diseases and problems with the control of dietary intake.

A uremic animal model may simulate the clinical situation and provide more knowledge of adequate nutritional treatment in chronic uremia. The main purpose of this study was to evaluate the effect of total parenteral nutrition (TPN) under standardized laboratory conditions in severe chronic renal failure in rats.

The study includes a comparison between two amino acid solutions, one composed of a mixture of essential amino acids and histidine, one with a mixture of essential and non-essential amino acids; also two oral diets, one with a high and one with a low nitrogen content (24% and 6% protein diet). As a high energy supply is often indicated in CRF (9, 10) the effects of administration of fat in TPN was also studied.

Methods

Animals. Male rats (Wistar strain, Møllegaard, Copenhagen, Denmark) weighing 160-180 g were made uremic by partial resection and irradiation of the left kidney followed by contralateral nephrectomy 5-7 days later as described in detail by Sterner (11). Control rats were sham-operated. Permanent catheterization of vena cava superior via vena jugularis externa was performed according to a method described by Roos et al. (12) in connection with the second operation in both uremic and sham-operated rats. All rats were kept individually in a controlled environment (temperature $+23^{\circ} \pm 1^{\circ}$ C, relative humidity $60 \pm 10\%$, and light from 6 a.m. to 6 p.m.).

Experimental diets and test period. The rats were fed laboratory stock diet (R3 Astra Ewos, Södertälje, Sweden) with 24% protein as soy meal and crude fish protein and water ad libitum, until the start of the experimental period on day 10 after the second operation. From day 10 to 20 the rats were given different nutritional treatments:

a) 17 uremic and 12 sham-operated rats were given TPN according to Table I. The nitrogen level used during TPN was 0.6 g N/kg/day either as essential amino acids + histidine (13), EAA (Aminess[®], KabiVitrum, Stockholm, Sweden) or as a complete mixture of essential and non-essential amino acids, CAA (Vamin[®], KabiVitrum, Stockholm, Sweden). Maintenance level of energy intake in rats was determined by Tao et al. (14) to approximately 1090 kJ/kg/day using i.v. infusion of amino acids and glucose. Increased energy intake has been recommended in the uremic state (9) and a total supply of 1385 kJ/kg/day was therefore chosen in the TPN study.

TABLE I. Amounts of nutrients given per kg bodyweight and day during TPN.

Component	g/kg/day	ml/kg/day	Preparation
Nitrogen	0.6	65	CAA [*]
		100	EAA ^{**}
Glucose	61-63	113-116	Glucose 50%
Triglycerides	9		
Egg yolk phospholipids	0.55	46	Intralipid 20%
Glycerol	1.0		

Amount of amino acids in the solutions (g/l).

* CAA = Ile 3.9, Leu 5.3, Lys 3.9, Phe 5.5, Tyr 0.5, Met 1.9, Cys 1.4, Thr 3.0, Trp 1.0, Val 4.3, His 2.4, Ala 3.0, Arg 3.3, Asp 4.1, Glu 9.0, Gly 2.1, Pro 8.1, Ser 7.5.

** EAA = Ile 5.3, Leu 8.3, Lys 6.0, Phe 8.3, Met 8.3, Thr 3.8, Trp 1.9, Val 6.0, His 4.1.

Total energy supply: 1385 kJ (330 kcal)/kg bodyweight/day

Total volume supply: 280 ml/kg bodyweight/day

Minerals and vitamins added to 1000 ml of TPN solution:

Na 1340 mg, K 1322 mg, Ca 365 mg, Mg 107 mg, P 290 mg, Cl 2730 mg, Fe 4 mg, Mn 0.5 mg, Cu 0.16 mg, Zn 4.85 mg, Co 0.025 mg, Mo 0.05 mg, Sn 0.015 mg, I 0.3 mg, F 1.0 mg, Se 0.008 mg, S 100 mg (Vamin[®]) and 80 mg (Aminess[®]) 50% of sulphur in met and cys plus 10 mg as sulphate in Vamin[®], Vit. A 2700 IU, Vit. D₃ 70 IU, Vit. E 18 IU, Vit. K₁ 0.14 mg, Vit. B₁ 2.5 mg, Vit. B₂ 2.5 mg, Niacinamide 1.5 mg, Vit. B₆ 4 mg, Vit. B₁₂ 0.004 mg, panthotenic acid 1.5 mg, folic acid 1.5 mg, biotin 0.1 mg, Vit. C 5.5 mg, Choline 270 mg, PABA 13.5 mg, Inositol 27 mg.

b) A complete 6% egg protein diet (Table II) was fed ad libitum to 11 uremic and 7 control rats. As Table III shows the nitrogen and total energy intake was 0.6 g N/kg/day and 1350 kJ/kg/day for both groups of rats.

c) A diet containing 24% protein (described above), was given ad libitum to 7 uremic and 8 control rats. Intake in both groups was 2.9 g N/kg/day and 1200 kJ/kg/day (Table III). The day before the start of the test period the orally fed rats were placed in two types of cages specially made for oral balance studies.

Infusion procedure. All rats given infusions were placed individually in metabolic cages that allowed the rat to move freely (12). Intravenous infusions were given 24 hours/day via the permanent catheters at a controlled rate by means of infusion pumps (Holter 904, Extracorporeal M.S. Inc., King of Prussia, Pa., USA). Fresh solutions were aseptically mixed every day under laminar air flow. Specially composed vitamin and mineral additive solutions (Table I) were used to provide a complete nutrition programme adapted to the requirement of the rat. The final mixing of all the solutions was done in sterile 300 ml infusion bottles (one for each rat and day). The ad libitum fluid intake in the uremic rats was found to be 250 ml/kg/day in a limited test. However, to fulfil the nutritional regimen with the available solutions, it was not possible to give less than 280 ml/kg/day.

Weight increase and nitrogen balance. The rats' bodyweight and general appearance were noted every day and the rats were then disconnected for a few minutes from the infusion set. The mean weight increase per day during the test period was calculated excluding the first three days, as body weight can be influenced by changes in environment, general nutrition, and dietary amino acid pattern (15). The recorded bodyweight on the last day after completed infusion was also excluded, as the noted decrease in body weight probably was due to a different weighing time. Urine and faeces from rats given the different nutritional regimens were collected quantitatively each day from day 4 to day 10, homogenized and analysed according to a micro-Kjeldahl technique (16), and nitrogen balance was calculated.

TABLE II. Composition of the 6% egg protein diet.

Component	g/kg diet
Egg albumin powder	60.0
Wheat starch	268.9
Sucrose	268.9
Glucose	268.9
Soybean oil	50.0
Vitamin oil a)	10.0
Vitamin mix 1 b)	5.0
Vitamin mix 2 c)	5.0
Mineral mixture d)	53.3
Cellulose powder	10.0

- a) Vitamin-A palmitate 20 000 IU; Vitamin D₃ 2000 IU; alpha-dl-tocopherol, 100 mg dissolved in 10 g of soybean oil.
- b) 5.0 g of vitamin mix 1 contain: Choline chloride 2.0 g and sucrose 3.0 g.
- c) 5.0 g of vitamin mix 2 contain: Thiaminmononitrate 5 mg, riboflavin 8 mg, pyridoxin HCl 5 mg, niacinamide 40 mg, calciumpantothenate 40 mg, P-amino-benzoic acid 2 mg, biotin 0.4 mg, folic acid (pteroyl glutamic acid) 2 mg, vitamin B₁₂ 30 µg, inositol 100 mg, menadione 5 mg and sucrose to 5 g.
- d) 53.3 g of mineral mix contain: NaCl 6.97 g, KH₂PO₄ 19.46 g, MgSO₄ x 7 H₂O 5.87 g, CaCO₃ 19.08 g, FeSO₄ x 7 H₂O 1.35 g, MnSO₄ x H₂O 0.20 g, ZnSO₄ x 7 H₂O 82 mg, CuSO₄ x 5 H₂O 24 mg, CoCl₂ x 6 H₂O 1 mg, Na₂SeO₄ x 10 H₂O (1/1000 with sucrose) 234 mg, KI 39 mg.

TABLE III. Intake of nitrogen, energy and fat in orally fed rats during the test period (mean $\pm$ SE).

	6% protein diet*		24% protein diet**	
	Uremic rats (n=11)	Control rats (n=7)	Uremic rats (n=7)	Control rats (n=8)
Intake of nitrogen g/kg/day	0.63 $\pm$ 0.03	0.61 $\pm$ 0.04	2.87 $\pm$ 0.09	2.89 $\pm$ 0.10
Intake of energy kJ/kg/day (metabolisable energy)	1350 $\pm$ 60	1330 $\pm$ 90	1190 $\pm$ 40	1200 $\pm$ 40
Intake of fat g/kg/day	5.1 $\pm$ 0.2	5.0 $\pm$ 0.3	4.0 $\pm$ 0.2	4.1 $\pm$ 0.1

* The 6% protein diet was a complete semisynthetical powder diet with egg albumin as protein source (average nitrogen content 0.74%). Energy content was estimated to 16 MJ per kg diet with heat combustion and corrections for a digestibility of 98% and specific dynamic action (Atwater's factor).

** The 24% protein diet was laboratory stock diet (R3, Astra Ewos, Sweden) with energy content of 16 MJ per kg diet, crude protein 24% (3.85% N), carbohydrate 490 g and fat content of 54 g per kg.

The utilization of nitrogen was calculated according to the following formula:

$$\text{NNU}_{\text{op}} = \frac{\text{given N} - (\text{F} + \text{U}) + \text{E}}{\text{given N}} \times 100$$

NNU_{op} = Net Nitrogen Utilization, operative value

Given N = Supplied nitrogen in the test group

F = Faeces

U = Urine

(F+U) = Total excretion of nitrogen in the test group

E = Endogenous excretion of nitrogen. This figure was based on the physiologically active body weight according to Mitchell (17).

Biochemical data. Blood samples were obtained from the tail vein of the rat on day 0 (1.0 ml), day 2 (0.4 ml), and day 10 (0.4 ml) of the test period and analysed for serum-creatinine, plasma-urea, and packed cell volume (PCV). One to two hours after completed experimental period (day 10) blood was collected by cardiac puncture and serum analysed for sodium, potassium, chloride, calcium, phosphate, osmolality, cholesterol, and albumin, and blood for hemoglobin. Plasma-urea was determined in an automatic amino acid analyzer (Durrum D-500, Dionex Corp., Sunnyvale, Calif., USA). Serum analysis was performed by routine methods (Medilab AB, Stockholm, Sweden). Hemoglobin was determined with the cyanmethemoglobin method on whole blood. On some of the uremic rats, 24 hours' creatinine clearance was measured during the day before test start and on the last infusion day. Creatinine analysis in urine was performed according to a modified Jaffé-method (18).

Statistics. Differences were calculated with the Mann-Whitney rank sum test and Wilcoxon's test for paired data and considered significant when P-values less than 0.05 were obtained (19).

Results

Altogether, 17 of 25 uremic and 12 of 17 sham-operated rats completed the TPN period. Three uremic rats died during the first days of infusion with anuria; four rats completed 8-10 days of infusion, but died before the termination of the experiment. Five sham-operated and one uremic rat did not complete the TPN period due to catheter obstruction or leakage.

Growth and nitrogen balance. Table IV and Figure 1 give the growth, nitrogen balance, and utilization in the four uremic groups and also in the four sham-operated groups. The control rats fed the low protein diet lost about 20 g and the uremic rats 10 g during the 24 hours before the start of the test period. The metabolic cages used for these groups hampered normal food intake initially. The mean growth rate lay between 1.8-3.0 g/day for the uremic groups and 3.1-3.3 g/day for all sham-operated groups. Weight increase did not differ significantly either between the two amino acid solutions given in TPN or between uremic and sham-operated rats in this group. TPN with EAA in uremic rats gave significantly better gain in weight than the 6% oral diet ($p < 0.05$). The growth rate for uremic rats with oral 6% and 24% protein diets was significantly lower than the corresponding sham-operated groups ($p < 0.05$). The nitrogen balance was positive in all uremic and sham-operated groups with values of 40-74 mg /day. In rats given TPN with CAA, the nitrogen balance was less positive in the uremic group than in the sham-operated group ($p < 0.01$). Otherwise no significant difference was found.

The nitrogen utilization in the rats given TPN was 72-75% for the uremic group and approximately 84% for the controls. Uremic rats given TPN with CAA, but not rats given TPN with EAA, differed significantly compared to sham-operated rats ($p < 0.05$). Control rats on TPN utilized the nitrogen significantly better than rats fed the low nitrogen diet. The 24% protein fed uremic rats had a low degree of nitrogen utilization, but the intake of nitrogen was far higher in this group.

TABLE IV. Weight gain, nitrogen balance, and net nitrogen utilization in uremic (U) and control (C) rats given TPN, 6% protein, and 24% protein diets (mean; range).

		Weight gain g/day	N-balance mg/day	Net nitrogen utilization %
TPN with CAA	U (n=9)	2.72 (1.02-4.68)	+44 (+12 - +58) ^a	72.1 (45.4 - 81.2) ^{b,e}
	C (n=6)	3.17 (1.62-4.27)	+73 (+57 - +87)	84.8 (73.6 - 94.7) ^c
TPN with EAA	U (n=8)	2.98 (1.95-4.20) ^d	+56 (0 - +87)	74.7 (46.9 - 91.0) ^e
	C (n=6)	3.25 (1.02-3.55)	+74 (+56 - +93)	83.7 (79.6 - 93.0) ^d
6% protein diet	U (n=11)	1.81 (0 - 3.80) ^b	+40 (+15 - +66)	54.6 (0 - 75.1) ^f
	C (n=7)	3.13 (1.80-4.05)	+45 (-11 - +77)	62.1 (26.6 - 81.6)
24% protein diet	U (n=7)	1.85 (0.42-3.28) ^b	+72 (-102 - +145) [*]	25.4 (0 - 34.0)
	C (n=8)	3.16 (1.10-4.03)	-----	-----

a significant difference to control p<0.01 d significant difference to 6% diet p<0.05

b " " to control p<0.05 e " " to 24% diet p<0.01

c " " to 6% diet p<0.01 f " " to 24% diet p<0.05

* nitrogen balance performed on a separate group (n=8)

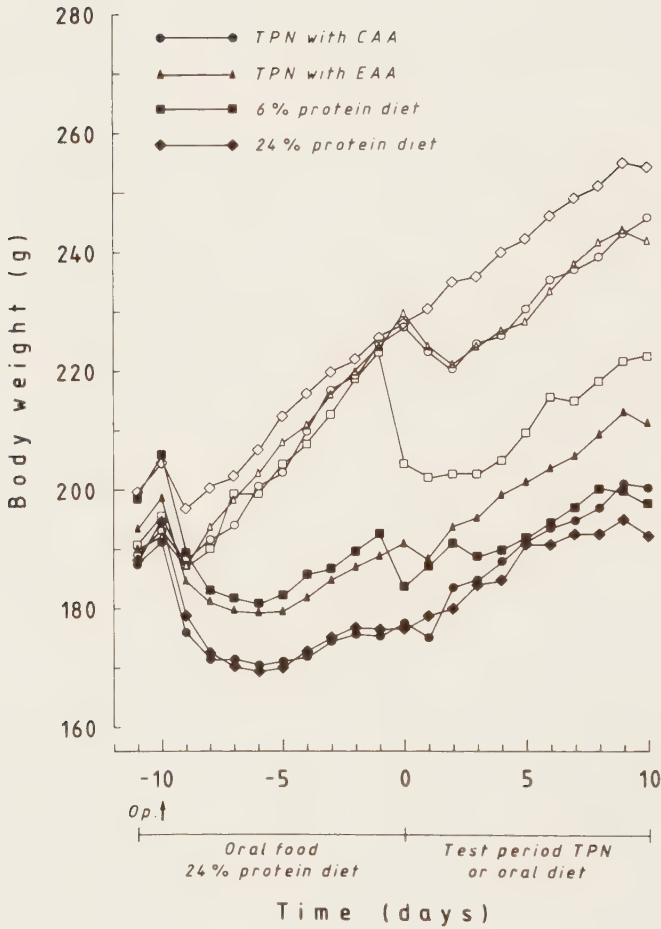


Fig. 1. Weight increase graph for uremic and sham-operated rats given TPN with EAA or CAA continuously (0.6 g N/kg/day) during a 10 days' period, compared to oral 24% and 6% protein diets (mean values). Filled symbols represent uremic animals and open symbols non-uremic controls.

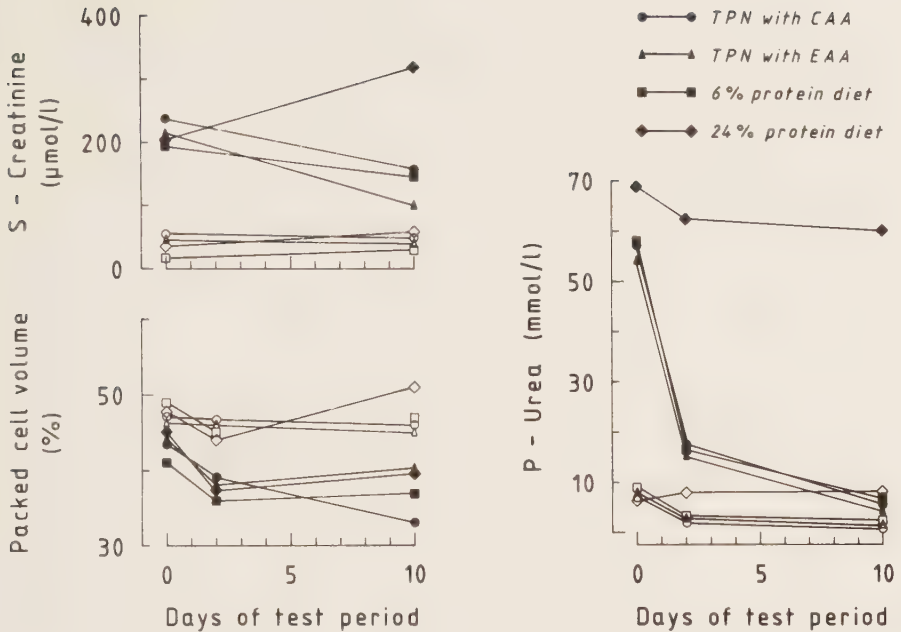


Fig. 2. Plasma-urea, serum-creatinine, and packed cell volume in rats during TPN and oral nutrition (mean values). Filled symbols represent uremic rats and open symbols non-uremic controls.

Urea and creatinine levels. Plasma-urea levels (Figure 2) were considerably elevated in the uremic rats (59.1; 23.5-120.0 mmol/l for the whole group, mean; range) compared to the controls (7.8; 5.1-11.4) on day 0 ($p < 0.01$). Urea levels dropped markedly throughout the study, especially during the first days, in all groups given TPN (5.2; 1.9-16.0 and 1.2; 0.6-3.6 for uremic and sham-operated rats on day 10). There was no difference between rats given TPN with CAA and EAA on day 10 of the infusion period. Urea levels in the 6% protein fed uremic group were significantly reduced (7.0; 3.0-13.8 day 10, $p < 0.01$), whereas the values in the 24% protein uremic group were high and stable during the test period. Plasma-urea values were decreased in the sham-operated rats given the 6% protein diet to a level similar to the TPN control groups (1.8; 1.1-2.5 on day 10).

Serum creatinine concentrations (Figure 2) were increased 5-fold in the uremic rats compared to sham-operated controls on day 0 ($p < 0.01$). Significant differences were also observed on day 10

($p < 0.01$) but levels decreased in the uremic groups during TPN ($p < 0.01$). No significant change was noted in uremic rats fed the 6% protein diet while the 24% protein diet resulted in increased serum creatinine ($p < 0.05$).

Clearance of creatinine was measured in some of the uremic rats. A decrease was noted in uremic rats fed the oral diet (24% protein $n=3$, 6% protein $n=9$), significant for the 6% protein diet ($p < 0.05$). Creatinine clearance in uremic rats given parenteral nutrition was similar on day 0 and day 10 (CAA $n=4$, EAA $n=3$).

Remaining laboratory data. As Figure 2 shows, packed cell volume (PCV) was normal on day 0 in the uremic groups and dropped 10-15% during the first days. On day 10, there was significantly lower values for uremic rats given TPN with CAA ($p < 0.05$), oral 6% protein diet ($p < 0.01$) and 24% protein diet ($p < 0.05$) compared to non-uremic controls. PCV did not significantly differ between uremic and sham-operated rats given TPN with EAA. No changes were noted in the control groups during the study.

Serum values after the experiment for sodium, potassium, chloride, phosphate, calcium, osmolality, cholesterol, albumin, and hemoglobin are listed in Table V. No deviations in electrolyte concentrations resulted from the total parenteral nutrition in either uremic or sham-operated rats. Sodium concentration was increased in uremic rats fed the 24% protein diet compared to the other uremic groups ($p < 0.05$). Serum osmolality was normal in all groups except for elevated levels in the uremic rats on the 24% protein diet ($p < 0.01$). Serum cholesterol was higher in uremic groups given the two oral diets ($p < 0.01$) than in controls. This difference was not found after administration of TPN with fat. Shown from the same Table V serum albumin and hemoglobin values on day 10 were significantly decreased in all uremic groups compared to sham-operated controls.

Discussion

This study shows that total parenteral nutrition can be performed in severely uremic rats. We know of no earlier reports on total parenteral nutrition given to chronically uremic animals, although the effect of amino acid infusions in acute renal failure have been studied in rat (20,21) and dog (22,23).

The technique for administration of TPN was well applied to the uremic rat model used. The serum electrolytes analysed after TPN did not deviate from normal rat and no marked signs of overhydration, as oedema and ascites, were seen. The period of stable creatinine and urea levels from day 10 to 20 was chosen for the study and no deterioration of kidney function was found after TPN. The problems with achieving a correct measure of nitrogen balance in animals, otherwise well known (24), were minimized with the application of TPN as food contamination of urine and faeces was avoided. Another method of assuring a controlled food intake is tube feeding (25) although this method is possibly more stressful to the animals.

The low amino acid supply via TPN resulted in positive nitrogen balance and weight gain after a few days and significant decrease in serum urea and creatinine. We did not find any difference between the two amino acid solutions given in TPN to uremic rats as far as weight gain and nitrogen utilization was concerned.

The uremic and control rats on 6% protein diet showed equally positive nitrogen balances but differed significantly in weight gain. Thus it appears that the retained nitrogen was not used for growth to the same degree in the uremic state as in normal rats given low protein food. Changes in urea levels after the second day of the experiment were too small to account for these discrepancies.

The low degree of nitrogen utilization in the 24% protein fed rats is most probably an effect of the high nitrogen intake. It is known from oral feeding studies with proteins of different qualities that the efficiency of protein utilization decreases with increasing protein levels in the diet (26,27).

Earlier reports have been concerned with the role of oral diets with different protein content given to uremic rats (25,28,29). Wang et al. (29) found that 15% and 23% protein diet gave approxi-

mately the same weight gain, whereas weight increase after 5% protein diet was less. All uremic groups in this study showed lower weight gain per gramme of protein consumed than the corresponding non-uremic rats. Pennisi and co-workers (25) administered isocaloric diets by stomach tube to uremic rats to ensure adequate food intake. They found that either casein or a mixture of amino acids was superior to an isonitrogenous solution of essential amino acids in promoting a good nutritional status. Their conclusion was that non-essential nitrogen might be limiting and that treatment with only essential amino acids was not optimal. On the contrary, our study shows that a low nitrogen dose administered as essential amino acids in TPN results in adequate nitrogen balance and weight increase. There is nothing indicating a better nutritional status when all amino acids were supplied (CAA) at this low nitrogen level.

Albumin and hemoglobin levels were reduced significantly in all uremic rats compared to controls and was not influenced by the different nutritional treatments. The effect of a longer dietary treatment is not known. In a comparable study over 3 months, Young et al. (30) however, found hemoglobin and albumin to be dependent on protein content in the oral diets.

Several authors (9,10,25,29,31,32) have suggested that a low food intake is a contributing factor to the wasting seen in chronic uremia. However, the uremic rats fed the oral diets in our study had a food intake equal to controls but still gained less in weight which confirms the data obtained by Young et al. (30). Equally uremic rats given TPN with the same intake of nitrogen and slightly higher intake of energy than the 6% protein group showed a weight increase comparable to control rats. Possible explanation for this disparity is small intestinal dysfunction in uremia (33) or the continuous administration and the composition of nutrients in TPN. The TPN regimen used in this study contained nearly twice as much fat as the oral diets with no adverse effects on serum cholesterol. Energy administered as fat is possibly better utilized than carbohydrate in the uremic state, as there is evidence of altered carbohydrate metabolism with decreased tissue sensitivity to insulin in uremic man (34). On the other hand, hypertriglyceridemia and reduced catabolism of triglycerides is common in uremia (35), but this was not studied.

To summarize: we have found that parenteral nutrition in this uremic rat model can be performed with good results on the general condition of the animals. Both amino acid solutions promoted positive nitrogen balances and weight gain comparable to controls. In uremic rats TPN, including a fat emulsion, gave a growth rate superior to that seen with oral feeding. This experimental model of chronic renal failure simulates the uremic state seen in patients with CRF. It can therefore be useful for further studies on nitrogen and energy requirements in uremia and the effects of different amino acid and ketoacid supplementation.

Acknowledgements

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VI

PLASMA FREE AMINO ACID LEVELS IN UREMIC RATS GIVEN HIGH AND LOW PROTEIN DIETS OR INTRAVENOUS INFUSIONS OF AMINO ACID SOLUTIONS

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Abstract

Plasma free amino acids were measured in uremic and sham-operated rats fed for 10 days a high (24%) or a low (6%) protein diet or given total parenteral nutrition at a low nitrogen level. The parenteral nutrition supplied either a complete amino acid solution or a solution containing only the essential amino acids.

Irrespective of dietary protein level or composition of the amino acid solution administered, increased levels of citrulline, glycine, hydroxyproline, and 1- and 3-methylhistidine were found in uremic rats. Regarding the essential amino acids, several differences were seen after oral feeding, but few after intravenous nutrition. Likewise was the marked reduction in plasma tyrosine found only in the orally fed groups. The tyrosine: phenylalanine and citrulline: tryptophan ratios were found to depend mainly on the uremic state whereas the nitrogen intake and uremia influenced the valine: glycine and the essential: non-essential amino acid ratios.

The alterations observed in the plasma free amino acid levels and ratios of the uremic rats were similar to those reported from clinical observations on patients with chronic renal failure.

Introduction

In patients with chronic renal insufficiency certain deviations from the normal free amino acid pattern in plasma and muscle have been observed (1-13). Similar changes have also been noted following the experimental induction of chronic uremia in animals (14-19). It is not clear to what extent these modifications in the plasma free amino acid levels can be explained by protein malnutrition, by hormonal imbalances or by specific changes in the retention or metabolism of certain amino acids.

The present paper gives an account of the concentrations of plasma free amino acids (PAA) noted during the oral feeding of high (24%) and low (6%) protein diets and after the intravenous administration of two different amino acid solutions in an uremic rat model system. One of the infused solutions was complete with 13 amino acids; the other contained only the 9 amino acids considered essential in uremia (20-22). The amino acids were given for

10 days as part of a total parenteral nutrition (TPN) schedule, including a fat emulsion. The experimental set-up and the results concerning growth, nitrogen balance, and certain biochemical parameters are described in more detail elsewhere.¹

Material and Methods

Uremic model and experimental procedure

Male Wistar rats², weighing 160-180 g, were subjected to extensive resection and irradiation of the left kidney followed by contralateral nephrectomy about one week later (23). Control rats were simultaneously sham-operated and their left kidney irradiated. In conjunction with the second operation, a permanent catheter was inserted in the vena cava superior (24) of the uremic and also the sham-operated rats. This technique produces rats with severe uremia. In a preliminary test, we found urea and creatinine levels to be stable between days 10 and 20 after the contralateral nephrectomy. This period was therefore chosen for the experimental investigation. Table 1 gives a comprehensive review of the planning schedule. Several series of experiments were carried out, each study generally containing a few animals from each dietary treatment.

Experimental diets and infusions

During the days preceding the test period, the rats were given a commercial pellet diet³ with a crude protein content of 24% and tap water ad lib. At the beginning of the experiment (day 0), both sham-operated and uremic rats were divided into 4 groups (for number of rats in each group, see Table 1) and given one of the following dietary regimens:

¹ Wennberg, A., Sterner, G., Kihlberg, R. and Denneberg, T., Effects of total parenteral nutrition in rats with experimental chronic renal failure. To be published.

² Møllegaard, Copenhagen, Denmark.

³ R3-Brood stock fed for rats and mice, Astra Ewos, Södertälje, Sweden.

TABLE 1. Planning schedule for experiments with sham-operated (S) and uremic (U) rats.

Day	Approximately -16	Approximately -10	0	+10
Treatment	(S) First sham-operation	(S) Second sham-operation Catheter inserted	(S) and (U) Start of experimental period	(S) and (U) End of experimental period
	(U) Left kidney partly resected and irradiated	(U) Right kidney removed Catheter inserted	Blood sampling	Blood sampling
Nutritional treatment and number of rats per group	From arrival until start of experi- mental period	(S) (U) Oral feeding of diet containing 24% protein 27 33	Oral feeding 24% protein diet: 6% protein diet: Intravenous administration Complete AA-solution: Essential AA-solution:	(S) (U) 8 7 7 11 6 8 6 7

1. A high protein (HP) diet. Sham-operated (control) and uremic rats were fed ad lib. the same 24% protein diet as that given before the test period, based on cereals, fish protein concentrate and soy meal.³ Feed intake was not recorded for these rats but for two separate groups with 5 sham-operated and 8 uremic rats, respectively, fed identical diets.
2. A low protein (LP) diet containing 6% protein from egg albumin and also wheat starch, glucose, sucrose, and soybean oil and adequate in minerals and vitamins. The control and uremic rats were fed ad lib. and the intake measured.
3. A complete L-amino acid (CAA) solution,⁴ composed mainly according to the amino acid pattern of egg protein. The solution was administered by continuous intravenous infusion (24 hrs/day) at a low nitrogen level of 0.6 g N/kg b.w. and day, together with a 50% glucose solution (ex tempore), a 20% fat emulsion⁵ and additive solutions of vitamins and minerals. Total volume and energy supplied per kg body weight and day were 280 ml and 1385 kJ (330 kcal) respectively.

³ R3-Brood stock feed for rats and mice, Astra Ewos, Södertälje, Sweden.

⁴ Vamin®, KabiVitrum AB, Stockholm, Sweden.

⁵ Intralipid® 20%, KabiVitrum AB, Stockholm, Sweden.

4. An essential L-amino acid (EAA) solution,⁶ containing only the 9 amino acids considered essential for the uremic patient, was infused in control and uremic rats at the same nitrogen level as that used for the CAA-solution. All other nutrient solutions, total volume and total energy were equal to those of regimen 3 above.

The nitrogen level used for infusion of the CAA- and EAA-solutions was chosen to correspond as closely as possible to the amount of nitrogen provided by the LP-diet. Table 2 shows the feed intake and the calculated daily amino acid supply.

Analysis

Blood for analysis of urea and plasma free amino acids was collected from the tail vein in 100 μ l heparinized microcapillaries of glass. Samples were taken at approximately the same time of the day, before the infusion began on day 0, during infusion on day 2 and before the infusion ended on day 10. Blood samples from the orally fed groups were taken from nonfasted animals in the mornings of days 0, 2 and 10. (Only the levels of urea and 1- and 3-methylhistidine are reported for samples taken on day 2). The blood samples (300 μ l) were transferred to microtubes and centrifuged twice for 5 minutes at 9.500 g. The plasma was stored at -70° C until analyzed.

Plasma was deproteinized by the addition of sulphosalicylic acid (40 mg/ml plasma). Determinations of free amino acid concentrations were performed by ion exchange chromatography on a Durrum D-500 Amino Acid Analyzer.⁷ A procedure with five different Li^{+} -buffers and three changes of column temperature was employed.

Serum creatinine from day 0 and day 10 was analyzed by Medilab AB, Stockholm, Sweden, using a modified Jaffé-method (25).

Statistics

Significance of differences between control and uremic rats on day 0 (comprising about 30 rats/group) was tested with Student's t-test. The results are presented as means $\pm$ SE (Fig. 1).

⁶ Aminess®, KabiVitrum AB, Stockholm, Sweden.

⁷ Dionex Corp., Sunnyvale, Calif., USA.

TABLE 2. Approximate amino acid intake and amounts of amino acids infused (mg/day) in uremic rats during the test period.

Amino acid	G R O U P			
	HP	LP	CAA	EAA
Lysine	200	50	50	115
Threonine	12	40	40	75
Valine	170	55	55	115
Leucine	240	70	70	160
Isoleucine	125	55	50	105
Phenylalanine	140	55	70	160
Tryptophan	-	15	15	35
Methionine	70	35	25	160
Histidine	85	20	30	80
Alanine	180	50	40	0
Glycine	175	30	25	0
Serine	140	60	95	0
Proline	215	40	105	0
Arginine	210	50	45	0
Glutamic acid	600	100	115	0
Tyrosine	110	20	6	0
Aspartic acid	285	70	55	0
Cystine	60	25	20	0

Feed intake

	<u>g/day</u>	<u>g/day</u>	<u>ml/day</u>	<u>ml/day</u>
Sham-operated rats	20.3 ¹⁾	18.4 ²⁾	15.5 ³⁾	22.3 ⁴⁾
Uremic rats	13.9 ¹⁾	16.6 ²⁾	12.9 ³⁾	19.7 ⁴⁾

1) Stock diet, N=3.85%; 2) Egg albumin diet, N=0.74%,
 3) Complete amino acid solution, 9.4 g N/l; 4) Essential amino acid solution, 6.6 g N/l.

Differences between the other groups with a less number of animals were calculated using the Mann-Whitney rank sum test and were considered significant when P-values were <0.05 (26). Results are given as mean and range (Tables 3 and 4).

The difference in slope between the two regression lines of uremic and control rats, respectively, illustrating the correlation between plasma glycine and serine (Fig. 3), was tested by Student's t-test.

Results

Urea, creatinine and free amino acids in plasma at day 0

At the beginning of the test period, the uremic rats had significantly raised blood levels of urea and creatinine. Mean levels of p-urea and s-creatinine were approx. 59 mmol/l and 220 $\mu\text{mol/l}$, respectively compared with 8 mmol/l urea and about 40 $\mu\text{mol/l}$ creatinine for control animals.

Fig. 1 shows the plasma free amino acid levels of uremic and sham-operated rats at day 0. Of the essential amino acids, all but methio-

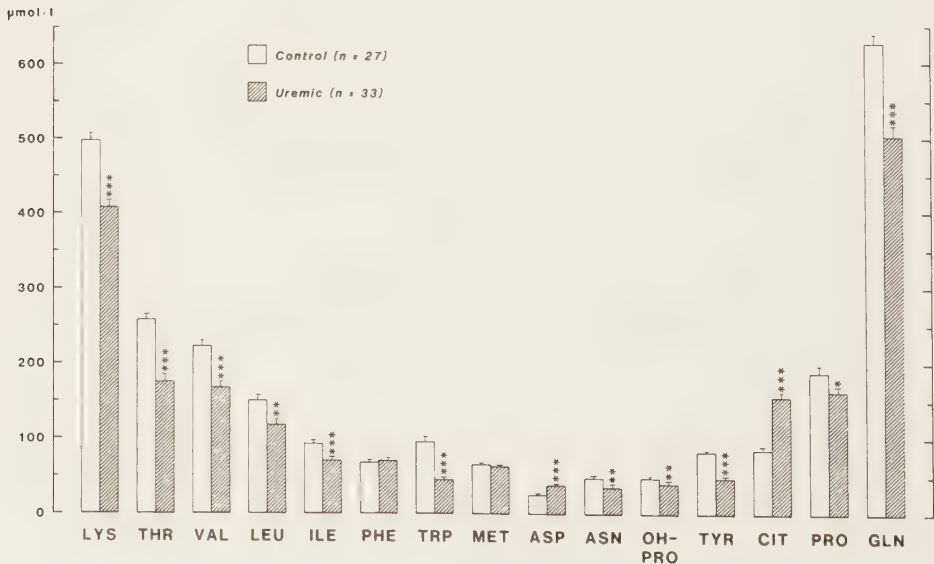


Fig. 1. Plasma free amino acid levels of control and uremic rats at the start of the test period (day 0). Mean values $\pm$ SE ($\mu\text{mol/l}$). Significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

nine, phenylalanine, and histidine were lower in plasma of the uremic rats. Larger concentrations were found for aspartic acid and citrulline while asparagine, glutamine, proline, hydroxyproline, and tyrosine levels were smaller in the uremic rats.

Feed intake and growth during the test period

As shown in Table 2 the feed intake of uremic rats fed HP and LP-diets was less than that of the corresponding control animals but calculated on a body weight basis, the intakes were similar (0.6 g N/kg and day for the two LP-groups and 2.9 g N/kg and day for the HP-groups). For control and uremic rats given CAA or EAA intravenously, the amount of nitrogen administered was equal. Increases in weight of uremic rats given LP- or HP-diets were lower than that of sham-operated rats on the same dietary regimen (approx. 1.8 versus 3.1 g/day). Control and uremic rats given TPN showed no significant differences in growth (mean values between 2.7 and 3.2 g/day).

Plasma free amino acid levels of uremic and control rats at day 10

Table 3 gives the plasma amino acid concentrations after the oral diets. Plasma levels of the branched chain amino acids (BCAA), threonine and tryptophan were still significantly lower in uremic than in sham-operated rats fed HP-diets. Tyrosine, glutamine, and serine were also significantly reduced in uremic rats whereas glycine, hydroxyproline, and citrulline levels were significantly increased. The differences in plasma levels of essential amino acids between uremic and sham-operated rats were less pronounced after the feeding of a LP-diet. However, valine, leucine, and tryptophan were lower in plasma of uremic animals as were the levels of tyrosine, and glutamine. Glycine and citrulline levels, on the other hand, were raised, together with aspartic acid, hydroxyproline, cystine, and taurine.

As shown in Table 4 no significant differences could be observed in the mean plasma levels of individual essential amino acids between sham-operated and uremic rats given TPN with a CAA-solution. However, higher levels were found in uremic plasma for glycine, serine, proline, hydroxyproline, alanine, aspartic acid, glutamic acid, cystine, and citrulline. After the infusion of an EAA-solution, only tryptophan of the essential amino acids was

TABLE 3. Plasma free amino acid levels of sham-operated and uremic rats orally fed for 10 days. Mean values ($\mu\text{mol/l}$), range, and significance of difference between uremic and control rats (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Amino acid	High protein diet (24%)				Low protein diet (6%)			
	Sham-operated rats (n = 8)		Uremic rats (n = 7)		Sham-operated rats (n = 7)		Uremic rats (n = 11)	
	Mean value	Range	Mean value	Range	Mean value	Range	Mean value	Range
Lysine	499.6	449-577	486.7	366-598	417.1	323-534	470.1	373-542
Threonine	278.0	240-311	214.4	170-280	129.7	108-180	124.9	74-249
Valine	291.8	254-356	157.1	125-201	175.6	129-199	145.6	96-177
Leucine	187.5	164-218	111.8	91-133	103.5	76-126	79.6	52-119
Isoleucine	113.9	103-130	76.6	61-94	66.6	47-78	63.9	43-73
Phenylalanine	75.4	69-83	72.0	62-82	58.7	40-82	68.0	54-85
Tryptophan	108.9	79-143	35.6	21-51	83.7	66-98	54.9	15-86
Methionine	71.6	80-59	79.3	65-94	46.1	41-50	51.9	32-88
Histidine	75.1	57-88	75.6	65-105	47.0	36-57	51.6	29-68
Glutamine	674.9	539-809	541.1	454-718	816.2	718-963	531.8	340-724
Alanine	403.7	324-446	491.3	330-637	748.7	650-966	805.4	557-1136
Glycine	225.3	212-332	389.6	291-487	669.3	561-812	1587.6	1001-2337
Serine	270.0	189-250	180.1	134-225	480.9	362-621	487.4	342-590
Taurine	313.3	187-527	349.5	258-558	367.4	180-517	487.4	275-621
Proline	241.0	199-292	216.9	140-286	204.3	182-229	217.4	146-267
Arginine	215.1	158-264	178.6	144-233	132.2	111-157	122.1	80-146
Citrulline	80.4	68-97	213.6	151-277	85.4	72-99	202.0	130-255
Glut.acid	77.71	52-103	82.32	68-106	76.0	65-105	66.0	49-79
Asparagine	47.51	43-59	33.22	16-40	64.3	51-83	52.0	31-68
Ornithine	76.3	60-113	66.2	39-95	53.2	40-72	48.9	36-63
Tyrosine	86.5	75-102	47.4	42-60	77.9	44-98	49.0	22-67
Hydroxyprol.	36.9	33-40	44.1	31-56	55.9	47-64	83.9	54-120
Asp.acid	36.0	21-49	43.2	28-60	30.9	20-49	43.5	26-65
Cystine	9.0	3-19	7.9	2-12	6.0	3-9	13.2	5-23

1) Based upon 6 values

2) Based upon 5 values

TABLE 4. Plasma free amino acid levels of sham-operated and uremic rats given total parenteral nutrition for 10 days. Mean values ($\mu\text{mol/l}$), range, and significance of difference between uremic and control rats (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Complete amino acid solution					Essential amino acid solution					
Amino acid	Sham-operated rats (n = 6)		Uremic rats (n = 8)		Sign. of diff.	Sham-operated rats (n = 6)		Uremic rats (n = 7)		Sign. of diff.
	Mean value	Range	Mean value	Range		Mean value	Range	Mean value	Range	
Lysine	349.6	294- 419	273.6	124- 736	NS	836.9	642-1050	1092.5	941-1253	**
Threonine	256.2	227- 298	286.8	217- 360	NS	561.0	507- 679	816.4	405-1216	NS
Valine	164.3	121- 192	175.7	141- 268	NS	206.5	148- 232	230.3	187- 345	NS
Leucine	83.8	66- 100	97.0	75- 191	NS	140.0	122- 154	161.4	92- 247	NS
Isoleucine	78.1	67- 87	85.5	62- 105	NS	90.7	73- 99	98.9	67- 153	NS
Phenylalanine	100.7	91- 109	115.2	94- 151	NS	147.7	122- 202	136.4	110- 185	NS
Tryptophan	65.1	39- 94	57.3	44- 70	NS	96.2	80- 133	69.8	59- 88	**
Methionine	61.2	56- 66	69.3	53- 87	NS	352.4	290- 414	347.4	314- 383	NS
Histidine	91.6	79- 102	98.8	88- 143	NS	172.7	140- 192	203.2	181- 233	**
Glutamine	813.4	675- 927	752.9	914- 597	NS	1336.2	928-1593	1218.9	1029-1449	NS
Alanine	662.7	478- 761	854.7	749- 994	**	387.4	299- 500	563.4	380- 966	NS
Glycine	973.3	641-1436	1994.2	1606-2484	***	268.4	180- 390	501.0	279- 781	*
Serine	676.6	526- 838	843.1	712- 993	*	247.6	177- 340	277.5	176- 492	NS
Taurine	271.4	139- 351	342.0	140- 552	NS	373.3	234- 551	680.3	409- 952	*
Proline	353.8	326- 385	458.1	362- 562	**	121.5	100- 165	168.1	137- 240	*
Arginine	102.5	78- 120	129.0	88- 227	NS	77.9	57- 109	94.6	53- 214	NS
Citrulline	36.6	10- 51	174.8	140- 213	***	64.4	53- 73	247.6	179- 313	**
Glut.acid	102.0	74- 157	178.0	157- 212	NS	87.2	66- 123	119.8	53- 176	NS
Asparagine	27.3	15- 40	44.6	33- 53	NS	47.3	34- 60	53.2	34- 80	NS
Ornithine	45.7	33- 70	55.4	39- 67	NS	28.8	22- 31	34.2	29- 52	NS
Tyrosine	67.5	56- 86	63.7	42- 98	NS	62.3	45- 87	98.2	63- 188	*
Hydroxyprol.	39.1	31- 49	62.4	46- 77	**	35.6	27- 42	63.6	45- 87	**
Asp.acid	41.3	33- 59	75.4	63- 90	***	33.4	26- 45	50.5	32- 59	**
Cystine	17.5	9- 27	28.8	20- 38	*	16.2	6- 36	21.1	14- 28	NS

1) Based upon 3 values

found to be less while lysine and histidine were larger in uremic rat plasma than in control rat plasma. The levels of 7 non-essential amino acids were significantly raised in plasma from the uremic rats.

Changes in certain plasma amino acids and PAA-ratios during the test

When the test period began (day 0), the total amount of free amino acids in the plasma of uremic rats was about 3 400-3 700 $\mu\text{mol/l}$ compared with 4 000-4 400 $\mu\text{mol/l}$ in control animals. Furthermore, the ratio of essential to non-essential amino acids (E:N ratio) was lower in uremic rat plasma at day 0; namely, 0.45 versus 0.57.

Based on data from the 8 groups at day 10, certain PAA-ratios were calculated (Fig. 2). A new ratio, citrulline to tryptophan was included, considering the profound changes of these amino acids in uremia.

Figure 2 shows that the E:N ratios of the uremic and the sham-operated groups given HP-diets were largely unchanged at the end of the test period. The introduction of a LP-diet resulted in marked and significant decreases in the E:N ratios in both uremic and sham-operated rats, but still the uremic E:N-ratio was significantly lower than that of the control group. In rats given TPN with CAA, the sum of non-essential amino acids increased substantially from day 0 to day 10. As a consequence, the E:N-ratio fell significantly in both the uremic and control group. The E:N-ratios of uremic and control rats on EAA-infusion increased significantly, in spite of considerable increase in the non-essential amino acid levels during the test period. The rise in both essential and non-essential amino acids was higher in the uremic than in the control rats.

As found in Figure 2 the valine:glycine ratio was markedly reduced by changing the dietary protein level from 24% to 6% both in the uremic and sham-operated groups. Uremic and control rats given EAA-solution intravenously had valine:glycine ratios 5 times larger than those of the uremic and control groups, respectively, infused with an isonitrogenous CAA-solution. The valine:glycine ratio of all uremic groups, was significantly less than that of the control groups on the same diet or intravenous regimen.

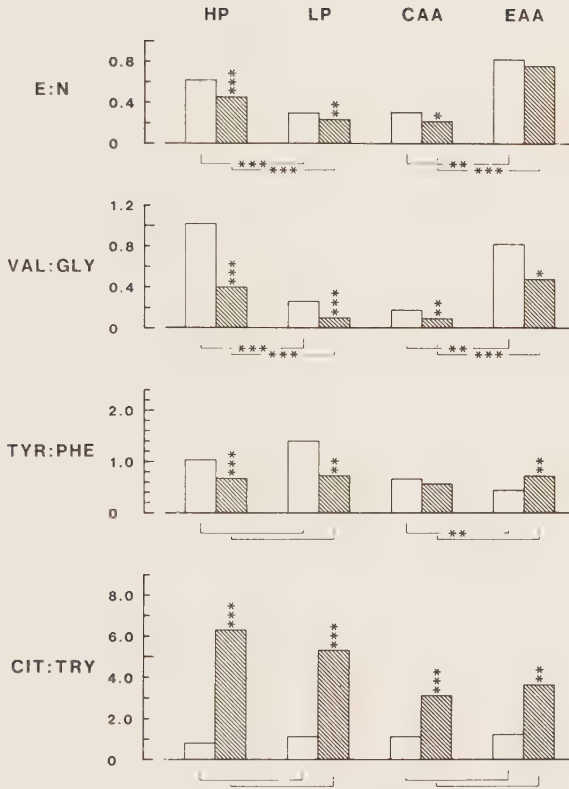


Fig. 2. Plasma amino acid ratios of control (□) and uremic (▨) rats given oral diets or TPN for 10 days.

HP: a 24% protein diet provided per os.

LP: a 6% " " " " "

CAA: a complete amino acid solution intravenously infused.

EAA: an amino acid solution with only essential amino acids intravenously infused.

Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Plasma phenylalanine levels did not differ between control and uremic rats on any of the nutritional regimens. Tyrosine levels of uremic rats orally fed HP and LP-diets were significantly less than those of the corresponding control groups. After intravenous administration of CAA, no differences in plasma tyrosine levels were seen. After EAA-infusion, tyrosine levels were larger in uremic than in control rat plasma. The ratio tyrosine: phenylalanine was significantly smaller in uremic than in sham-operated rats on oral diets but, in rats given EAA-solution, the ratio was significantly larger in the uremic group (Fig. 2).

Both arginine and ornithine increased in orally fed rats with increasing protein supply. There were no differences between control and uremic rats with respect to their plasma contents of arginine and ornithine. Citrulline, on the other hand, was significantly increased in all uremic groups but did not vary with changes in dietary protein intake. The citrulline:tryptophan ratio was significantly increased in all uremic groups compared with similarly nutreated control groups (Fig. 2).

There were no significant differences in plasma methionine levels between uremic and control rats. In the orally fed rats, plasma methionine was reduced with reduction of the dietary protein level. Infusion of the EAA-solution supplied about 7 times more methionine than the amount supplied by the CAA solution and the resulting plasma levels were increased 5 to 6 times in uremic and sham-operated rats.

In sham-operated rats, the plasma levels of 3-methylhistidine (3MH) were too small to be quantified. The uremic rats on the other hand, had plasma 3MH-levels around 20 $\mu\text{mol/l}$ at the beginning of the test period. The animals which continued on the high protein diet showed no changes in the plasma concentrations of 3MH during the test period. In the animals fed a low protein diet or infused with the two amino acid solutions at a low nitrogen level, however, the plasma 3MH levels sank rapidly. Already at day 2 the levels were significantly reduced by 35-50% in these three uremic groups. At day 10 the following values were recorded:

LP-group: 9.8 ± 0.8^8 ; CAA-group: 11.1 ± 0.8^9 ; EAA-group: 9.9 ± 1.2^{10} (mean $\pm$ SE, $\mu\text{mol/l}$). Similar changes were noted with regard to the plasma levels of 1-methyl-histidine (1MH).

Discussion

Some of the differences in our study between plasma amino acid levels of uremic and control rats were consistently observed, independent of dietary protein level or composition of the amino acid solution intravenously administered, e.g. increased levels of citrulline, glycine, hydroxyproline, and 1- and 3-methylhistidine in the uremic rats. Plasma tryptophan was reduced in 3 out of 4 uremic groups and the branched-chain amino acids and tyrosine were reduced in plasma of orally fed uremic rats but not after intravenous infusion. The ratios of plasma tyrosine to phenylalanine and citrulline to tryptophan were influenced mainly by the uremic state per se. The ratios of valine to glycine and essential to non-essential amino acids, on the other hand, were affected both by variations in nitrogen intake and amino acid composition and by the renal failure.

Our results are largely in good agreement with other studies on uremic rats (15, 19). In the experiments reported by Wang et al. (17) and Pennisi et al. (18), using different dietary treatments, the only consistent differences in plasma amino acid levels between uremic and control animals were increased levels of glycine and citrulline and, in the study of Wang et al. (17), decreased tyrosine:phenylalanine ratios.

Certain abnormalities in the plasma amino acid pattern of patients with renal failure are frequently reported as reviewed by Kopple et al. (27). Essential amino acids are generally decreased, particularly tryptophan and BCAA, whereas some non-essential amino acids, e.g. cystine, citrulline, 1- and 3-methylhistidine, glycine,

⁸ For technical reasons only 7 out of 11 samples were measured.

⁹ Eight out of 8 samples analyzed.

¹⁰ Five out of 7 samples analyzed.

and hydroxyproline are often increased. Tyrosine tends to be reduced as are the ratios of tyrosine:phenylalanine, valine:glycine, serine:glycine, and total essential:non-essential amino acids. The deviations in plasma amino acid pattern observed in our study are very similar to those reported for human subjects. This general agreement in experimental data suggests that our animal model system is suitable for studies concerning amino acid metabolism in chronic uremia. Some of the plasma amino acid deviations recorded are discussed in more detail below.

Tryptophan. More than 80% of total plasma tryptophan is normally bound to albumin (28). In animals with induced uremia and also in patients with renal failure, it is a common observation that the percentage of free tryptophan in plasma is increased whereas the total amount of plasma tryptophan is markedly reduced (15, 29, 30). It has been suggested that the increased concentration of indolic tryptophan metabolites in uremic subjects will increase the percentage and concentration of plasma free tryptophan by competing for binding to albumin (31).

The results from the present study suggest that two different mechanisms could be responsible for the reduction in total tryptophan level in uremia. The reduced tryptophan level in the uremic group fed a low protein diet and the absence of a difference between uremic and control rats during intravenous administration of a CAA-solution with a low tryptophan content suggest an impaired absorption. An impaired intestinal transport of amino acids in chronic uremia has also been suggested (32, 33). A second mechanism may be operating at high levels of tryptophan supply. Infusion of the EAA-solution with a tryptophan content 3 times larger than that of the CAA-solution resulted in a significantly lower level of plasma total tryptophan in the uremic rats compared with that of the control rats. An increased activity of liver tryptophan oxygenase in chronically uremic rats, as reported by Siassi and colleagues (34), would explain these differences.

The significance of the low plasma level of total tryptophan is not known. It has been hypothesized, however, that certain neurological symptoms commonly observed in uremia (35) might be associated with disturbances in the metabolism of the two neurotransmitter precursors, tryptophan and tyrosine (36).

Branched chain amino acids (BCAA). In our study a reduction of the dietary protein from 24 to 6% resulted in decreased BCAA levels in plasma of control and uremic groups. Similar results have been reported in non-uremic rats fed a low protein diet (37) and also in human subjects given diets with reduced protein contents (38, 39).

In the present study the concentrations of valine and leucine were significantly lower in plasma of uremic rats than in the control groups, fed the two oral diets. Low plasma levels of BCAA have been reported in some studies with experimental uremia (16) but not in other studies (15, 17). However, in patients with chronic renal failure, reduced levels of the BCAA seem to be consistently observed in plasma (27) and, concerning valine, also in the muscle free amino acid pool (10). Considering the suggested specificity of the BCAA in muscle protein metabolism (40), any reduction in the availability or change in the proportion of these amino acids could be of considerable importance.

In our study no significant differences in plasma BCAA levels were noted between uremic and sham-operated rats given intravenous infusions of CAA and IAA-solutions. Blood samples were taken during infusion, but at a stage when a steady state had been reached, and thus any differences in degradation rate would be expected to affect plasma levels. Therefore it seems less probable that an increased degradation rate accounts for the low plasma BCAA concentrations generally found in uremia. This agrees with the decreased degradation rate of valine found by Jones & Kopple (41). A more plausible explanation for the reduced plasma BCAA observed after oral nutrition is a reduced intestinal uptake of these amino acids although no data to confirm this are yet available.

Glycine levels were significantly higher in all uremic groups than in the corresponding sham-operated groups. This is a consistent finding in tests with uremic rats (15, 17, 18), and in studies with chronically uremic patients (3, 5).

Increased plasma concentrations of glycine and serine were noted in young non-uremic rats fed a low protein diet (37) and raised glycine levels have also been observed after administration of low protein diets to children and adults (38, 39, 42). In our study, a reduction of the dietary protein level from 24 to 6% was found to increase free plasma glycine levels markedly both in control and

uremic animals. Also in the two groups infused with CAA-solution at a low nitrogen level, a marked rise in plasma glycine concentration occurred during the 10 day test period. Infusion of the EAA-solution, however, only raised glycine moderately in uremic rats.

Normal kidney is a major source for serine which is synthesized primarily from glycine (43). We also found that plasma serine levels paralleled the increased plasma glycine levels. A plot of the values, however, showed two distinctly different regression lines (Fig. 3). The uremic rats, irrespective of dietary treatment, have a smaller ratio of serine to glycine. A smaller serine to glycine ratio has also been noted by Kopple et al. (27), who suggested a reduced synthesis of serine from glycine in renal failure.

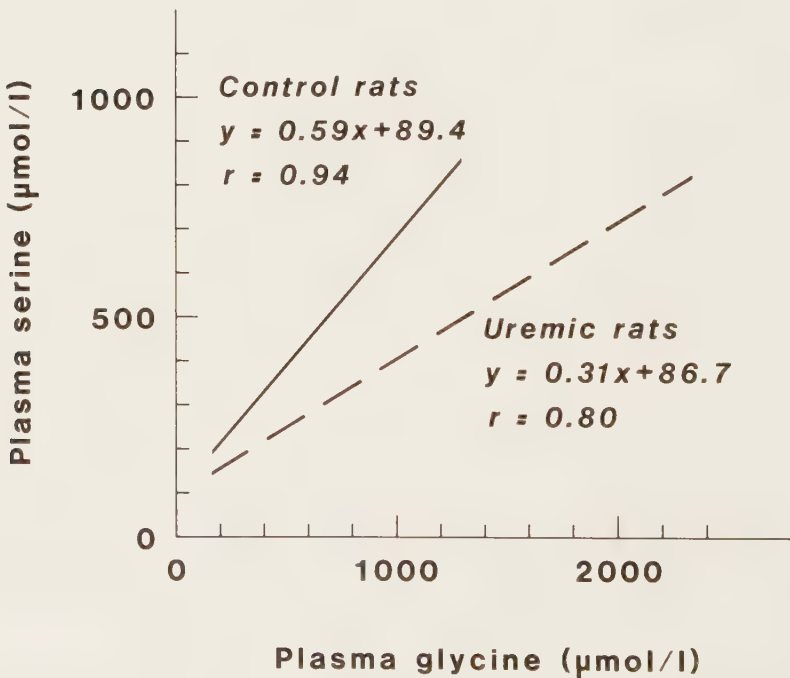


Fig. 3. Relationship between glycine and serine levels in plasma of control (solid line, $n=27$) and uremic rats (broken lines, $n=33$) given oral diets or TPN for 10 days.

Phenylalanine and tyrosine. Small plasma tyrosine levels and small tyrosine:phenylalanine ratios have been reported from animal experiments using chronic uremia models (15, 17) and also from studies of patients with chronic renal failure (27). Wang et al. (44) found the activity of phenylalanine hydroxylase in the kidney of chronically uremic rats to be reduced compared with normal rats. This might be of metabolic significance for the low tyrosine plasma levels found in the uremic state. In our study, uremic rats fed the oral diets had lower plasma tyrosine values, whereas uremic rats given EAA-infusion, containing no tyrosine, had significantly higher plasma levels than those of controls. There is no obvious interpretation of this observation. Uremic rats have possibly a reduced ability to metabolize or excrete increased quantities of tyrosine which might be formed from the comparatively large amount of phenylalanine in the EAA-solution.

The amount of methionine supplied by the EAA-solution was large and resulted in increased plasma levels of methionine in both groups and in the uremic group also in large plasma levels of taurine. Considering the suggested role of certain sulphur-amino acid metabolites in the development of vascular disease in patients with chronic renal failure (45) it might be advisable to avoid excessive administration of methionine in uremia.

Citrulline levels were strongly elevated in plasma of all uremic groups. This has been repeatedly observed in experimental uremia in rats (17, 19, 46) and also in studies with chronically uremic patients (2, 7, 11). It is known that the kidney can convert citrulline to arginine (47). A reduction in the arginine synthetase complex in kidneys of uremic rats was noted by Chan and colleagues (46), who suggested that a reduced kidney conversion of citrulline to arginine might explain the elevated citrulline levels in uremia.

Arginine is needed to support optimum growth of young rats (48). The uremic and control rats on EAA-infusion received no arginine which might have impaired the growth. In a separate study¹¹, no differences in growth were observed between two groups of young

¹¹ Bjelton, L. and Kihlberg, R., Personal communication.

non-uremic rats fed orally a mixture of essential amino acids at a low nitrogen level, with and without arginine. Thus, when dietary nitrogen level is low or the amino acid balance is inadequate, a dietary lack of arginine does not seem to affect the growth of the rats. It might still be questioned whether uremic rats, with a possible impairment in the synthesis of arginine by the kidney, would benefit from an exogenous supply of arginine. The studies of Wang and colleagues (49), however, indicated that the removal of arginine from an oral amino acid diet (corresponding to 18% protein), which reduced the growth of the control rats, did not affect the weight gain of uremic rats which was reduced with or without arginine.

There is no general agreement concerning the value of essential amino acids compared with a complete amino acid mixture in the treatment of severe uremia (50). The present study does not give the answer to this question. We compared the two solutions only on one nitrogen level, which does not allow a definite conclusion about their relative merits. On both solutions, despite the low nitrogen level, the rats grew well and showed a positive nitrogen balance. Plasma essential amino acid levels were considerably elevated in rats given the EAA-solution. The significance of this finding is not clear.

Considering the general agreement between our values and the clinically observed amino acid pattern, our rat model seems to be useful for studies concerning amino acid metabolism in uremia. However, in the present experiment, no direct comparison between plasma amino acid levels of orally and intravenously fed rats could be performed because the rats fed oral diets ate most of their food during the dark hours of the night whereas the rats on TPN were given 24 hours continuous feeding. Another approach, which could also provide information about intestinal uptake of amino acids in uremia, would be to give the same amino acid solution continuously by gastric and intravenous catheter. Additional analyses, such as determination of body composition and free amino acid pools in various organs, would add to the value of this animal model system.

To summarize: In this study of partially nephrectomized rats several alterations in the plasma concentrations and ratios of

free amino acids have been observed similar to those commonly seen in the clinical state of uremia. Certain differences observed between plasma amino acids of uremic and control rats after oral feeding were less pronounced after intravenous administration. Both dietary protein intake and uremia per se were important factors regulating the plasma amino acid levels.

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VII

SMALL INTESTINAL ABSORPTION OF GLYCINE AND GLYCYL- GLYCINE IN PATIENTS WITH CHRONIC RENAL FAILURE

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Abstract

Intestinal absorption of glycine and glycyl-glycine was studied in 9 patients with chronic renal insufficiency (mean creatinine clearance 9 ml/min) and 7 healthy controls. After an oral load of the amino acid or dipeptide, plasma alpha-amino nitrogen was repeatedly measured for two hours. In uremic patients, plasma alpha-amino nitrogen was significantly lower after glycine than after glycyl-glycine at 30, 45, and 60 minutes and also the area under curve was smaller after the amino acid indicating a reduced total uptake. One patient with a severe, terminal uremia had a flat curve after glycine administration. In control subjects plasma levels were lower after glycine than after glycyl-glycine at 30 minutes but the area under curve did not differ between the amino acid and the dipeptide. Generally these results agree with earlier studies in non-uremic man showing that, in small intestine, dipeptides are taken up better and by a different mechanism than amino acids.

Introduction

Amino acids and peptides of different length form the digestion products of dietary protein in the proximal jejunum. Earlier studies indicate different uptake mechanisms for amino acids and dipeptides in the small intestinal mucosa (Matthews 1975); it has been suggested that peptide uptake is of major importance in protein absorption (Matthews and Adibi 1976; Silk 1980).

Although gastrointestinal symptoms are common in uremia, actual studies on small intestinal function are scarce. We earlier found in an experimental model of renal failure in rat that uptake of amino acids (glycine, methionine, and lysine) were more depressed than the uptake of corresponding dipeptides in uremia (Sterner et al., in press). As far as we know no earlier investigation comparing amino acid and peptide absorption in the small intestine in uremia has been published. This paper reports the results from absorption studies on patients with chronic renal failure using oral tolerance tests with glycine and glycyl-glycine.

Material and Methods

We studied 9 patients (6 female, 3 male) with chronic renal insufficiency. Generally only patients with primary renal disease were accepted, although one patient (IH) was included with hydralazine-induced systemic lupus. None had been subjected to gastrointestinal surgery. Protein intake was approximately 40 g/day for most of the patients, but for some a higher intake was allowed. Antihypertensive drugs were given and phosphate binders, vitamin B, and folic acid were frequently added. A few received vitamin D in the form of $1 \alpha (\text{OH})\text{D}_3$; one (IH) was treated with low dose prednisolone. Most patients had a stable renal insufficiency with few uremic symptoms and were followed with 24-hours creatinine clearance regularly. However, one patient (LH) had a terminal uremia with a creatinine clearance of only 3 ml/min. Table I gives further data on the uremic patients.

Seven healthy subjects (3 female, 4 male) without any history of gastrointestinal and renal disease were used as controls (Table I).

The project was approved by the Ethical Committee of the University of Lund. All patients gave consent before participating in the study.

Both patients and controls were given glycine and glycyl-glycine as oral tolerance tests (Craft et al., 1968). The amino acid or the dipeptide was given, two to seven days apart, dissolved in 250 ml distilled water after an overnight fast. The quantity of glycine was 10 g per 70 kg body weight. Glycyl-glycine supply was 8.8 g per 70 kg body weight yielding the same number of glycine units as the glycine solution. Blood samples were taken before the start and at 15, 30, 45, 60, 90, and 120 minutes after giving either the amino acid or dipeptide. The patients and controls were recumbent during the experiments. Plasma was separated within two hours of the tests and kept frozen (-20°C) until analysed. Plasma α -amino nitrogen ($\alpha\text{-NH}_2\text{N}$) was determined with a ninhydrin-hydrindantin test as modified by Matthews et al. (1964). The reagents were kept under pure nitrogen gas to prevent reaction with ammonia in the air.

The increase over base-line level of $\alpha\text{-NH}_2\text{N}$ in plasma was plotted against time for all patients and controls. The area under

TABLE I. Data for the uremic patients and controls. CGN = chronic glomerulonephritis, CPN = chronic pyelonephritis, PC = polycystic disease, NSC = nephrosclerosis, SLE = systemic lupus erythematosus (hydralazine-induced), HN = hydro-nephrosis + renal artery stenosis.

Patient	Sex	Age (Y)	Weight (Kg)	Diagnosis	Serum creatinine ($\mu\text{mol/l}$)	24-hours creatinine clearance ($\text{ml/min}/1.73\text{ m}^2$)
IH	F	67	66	SLE	155	24
IJ	F	58	52	CPN	359	13
AN	M	58	69	CGN	744	11
AH	F	36	106	PC	670	10
MP	F	48	66	PC	686	9
AP	F	57	50	CGN	550	8
HL	M	73	62	NSC	853	8
NS	M	69	68	HN	882	7
LH	F	74	53	CGN	1144	3
Controls						
(mean)		50		74		
(range)		31-72		57-97		

curve (AUC) was calculated from 0-120 minutes using the trapezoidal rule. As the elimination half-time could not be estimated in all patients, no extrapolation of the curve to base-line was performed.

Glycine was kindly provided from Vitrum AB, Stockholm, Sweden and glycyl-glycine purchased from Sigma, St Louis, USA.

Statistics. Comparison between uremic patients and controls was performed with the Mann-Whitney rank sum test. Statistical differences within the uremic and control groups were examined with the Wilcoxon test for paired data. Differences were considered significant when a p-value less than 0.05 was obtained.

Results

Mean plasma level of α -NH₂N after an overnight fast was significantly higher ($p < 0.01$) for uremic patients (5.72 mmol/l; range 4.27-7.28) than for controls (4.37; 4.08-4.89).

Figures 1 and 2 give the results of the changes in α -NH₂N after an oral load of glycine or glycyl-glycine for each patient and control. Individual concentration curves varied considerably in uremic patients, particularly after the glycine test. Although no direct correlation between absorption of the amino acid and kidney function was found, several uremic patients had reduced uptake of glycine. Mean plasma levels of α -NH₂-N at 30, 45, and 60 minutes were significantly lower after glycine than after glycyl-glycine administration ($p < 0.05$). For control subjects a similar tendency was found although significant only at 30 minutes. AUC was lower after glycine than after glycyl-glycine in the uremic group ($p < 0.05$), whereas no difference was found in the control group.

No difference was noted between uremic and controls when comparing the glycine curves or the corresponding AUC-values. After glycyl-glycine administration, the peak value came later for the uremic patients than for controls. Mean α -NH₂N at 60, 90, and 120 minutes was significantly higher in the uremic group. However, AUC-values did not significantly differ after giving this dipeptide.

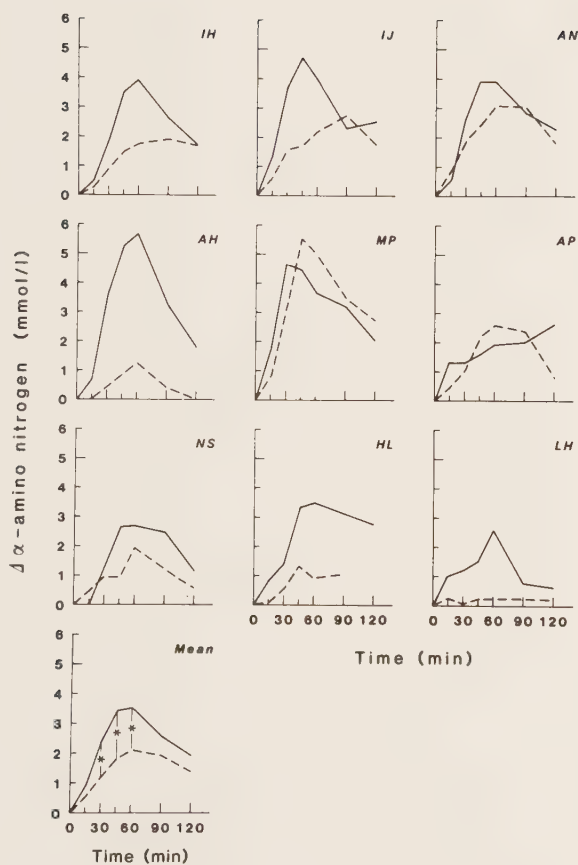


Fig. 1. Individual and mean increase of plasma α -amino nitrogen in uremic patients after oral administration of glycine (----) and glycyl-glycine (—).

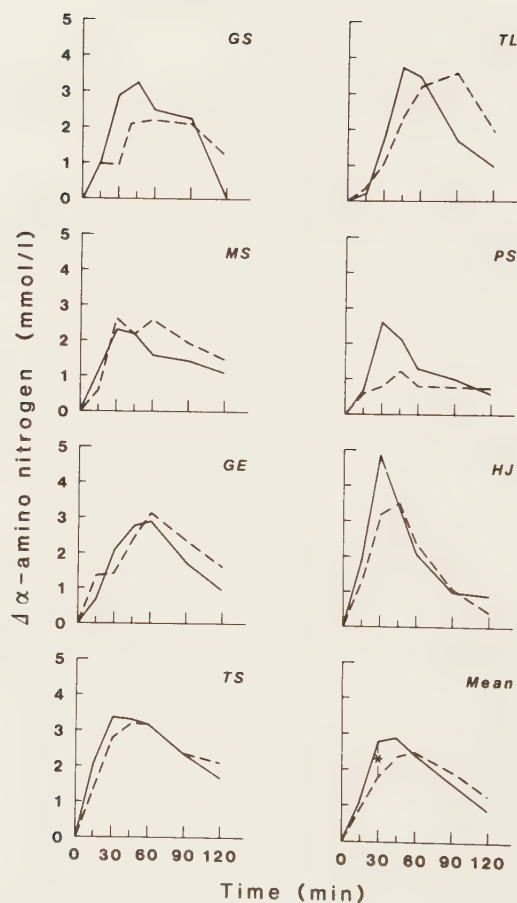


Fig. 2. Plasma α -amino nitrogen after oral administration of glycine (-----) and glycyl-glycine (—) to controls.

Discussion

Dietary treatment of patients with chronic renal failure (CRF) often includes protein restriction and oral supplementation with essential amino acids. Therefore information on digestion and absorption of protein and amino acids in uremia is important. This paper contributes to the knowledge of intestinal uptake function in patients with CRF, showing that absorption of glycine from the dipeptide is better than from equivalent amounts of free amino acid. Peak value in controls came faster and was only slightly larger after the dipeptide than after the amino acid. However, total uptake of glycine during the studied period did not differ from that of glycyl-glycine in the control group, as indicated by the similar AUC-values.

As the uptake of glycine was similar in the patients and control subjects, it seems unlikely that an important malabsorption of this amino acid occurs in patients with a stable renal failure without severe uremic symptoms. This is also suggested by the positive nitrogen balance achieved with oral amino acid supplement given to patients with CRF on a low protein diet (Bergström et al., 1975, Attman et al., 1979). However, one of our patients had a terminal uremia with severe gastrointestinal and mental symptoms. This patient's plasma glycine curve was flat indicating very low absorption of the amino acid, whereas uptake from glycyl-glycine was only slightly reduced. Thus it seems reasonable to suggest that the absorptive function could be impaired in patients with symptomatic uremia.

Previous literature is scarce concerning small intestinal absorption of amino acids in uremia. Gulyassy et al. (1972) found a lower and delayed plasma peak in patients with CRF than in controls after oral administration of tryptophan. They suggested that accelerated metabolism of tryptophan rather than decreased absorption was the cause of these results. Subsequent experimental studies in uremic rats have shown that activity of liver tryptophan oxygenase, responsible for breakdown of tryptophan, is increased in uremia (Siassi, 1975). Jones et al. (1978), though not primarily interested in intestinal absorption, studied plasma curves in patients with chronic uremia (creatinine clearance of 15 ml/min) after oral loading with phenylalanine. During the first 60 minutes, generally considered representative for the uptake phase, no

difference was noted when compared to controls with a normal kidney function. Later, plasma levels of phenylalanine rose higher in uremic patients, probably due to decreased conversion of phenylalanine to tyrosine.

Oral tolerance tests using glycine and glycine peptides were introduced by Craft et al. (1968). Their results from normal subjects were similar to ours with an earlier plasma peak value and significantly better uptake at 30 minutes using the peptide. It is now generally accepted that dipeptides are taken up by a different mechanism than amino acids in the small intestine in both man and experimental animal (Matthews and Adibi, 1976). Although oral tolerance test for measurement of intestinal uptake function is a simple method to accomplish, it has certain drawbacks. Gastric emptying and intestinal motility will affect the result, as will different distribution and metabolism of the amino acid after uptake. Segmental perfusion is a more direct way of measuring intestinal absorption. However, this technique also has many unsolved problems (Fordtran, 1966). In a separate experiment, we perfused solutions with either amino acids or dipeptides through a triple-lumen tube positioned in jejunum on 6 uremic patients with a mean creatinine clearance of 8 ml/min (Sternner, unpublished observation). We used a 10 cm mixing segment and a 30 cm test segment. Uptake was better from the dipeptide than from the amino acid solution but as the uptake during the mixing segment was substantial and varied considerably no definite conclusion could be drawn about the absorptive function in uremic patients compared with controls.

To summarize: This study shows that, in uremic patients, uptake of glycine from a dipeptide is better than from equimolar concentrations of free amino acids. In this way, the uremic intestine does not behave differently from the normal intestine. However, some patients have a very low uptake of glycine, whereas absorption of glycyl-glycine remains normal.

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